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H. W. WILEY, Chief of Bureau

PROCEEDINGS

OF THE

TWENTIETH ANNUAL CONVENTION

OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

HELD AT

WASHINGTON, D. C., NOVEMBER 19, 20, AND 21, 1903

EDITED BY

HARVEY W. WILEY

Secretary of the Association

WASHINGTON

GOVERNMENT PRINTING OFFICE

1904

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., March 4, 1904.

Sir: I have the honor to transmit herewith the manuscript of the proceedings of the twentieth annual meeting of the Association of Official Agricultural Chemists, with the request that it be published as Bulletin No. 81 of this Bureau.

Respectfully,

H. W. Wiley,
Chief of the Bureau of Chemistry and Secretary of the Association of Official Agricultural Chemists.

Hon. James Wilson,
Secretary of Agriculture.

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PROCEEDINGS OF THE TWENTIETH ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.

FIRST DAY.

THURSDAY—MORNING SESSION.

The twentieth annual convention of the Association of Official Agricultural Chemists was called to order by President R. J. Davidson at 10.15 o'clock on the morning of November 19, 1903, in the lecture hall of the Columbian University.

The following members and visitors were in attendance during the sessions of the convention:

MEMBERS AND VISITORS PRESENT.

Adams, Arthur B., Treasury Department, Washington, D. C.
Allen, William P., New Jersey Agricultural Experiment Station, New Brunswick, N. J.

Bache, A. W., U. S. Department of Agriculture, Washington, D. C.
Badger, E. F., State Board of Health, Providence, R. I.
Baskerville, Charles, University of North Carolina, Chapel Hill, N. C.
Beal, W. H., U. S. Department of Agriculture, Washington, D. C.
Benjamin, Marcus, Smithsonian Institution, Washington, D. C.
Bernays, Walter, City Chemist, St. Louis, Mo.
Bigelow, Willard D., U. S. Department of Agriculture, Washington, D. C.
Blake, F. E., U. S. Department of Agriculture, Washington, D. C.
Boyce, James, American Cotton Oil Company, Chicago, Ill.
Breazeale, J. F., U. S. Department of Agriculture, Washington, D. C.
Brooke, J. C., U. S. Department of Agriculture, Washington, D. C.
Brooks, R. O., 24 Jarvis place, Trenton, N. J.

Calm, C. E., 190 Michigan street, Chicago, Ill.
Cameron, F. K., U. S. Department of Agriculture, Washington, D. C.
Carpenter, F. B., Virginia-Carolina Chemical Company, Richmond, Va.
Cavanaugh, G. W., Cornell University Experiment Station, Ithaca, N. Y.
Chace, E. M., U. S. Department of Agriculture, Washington, D. C.
Chamberlain, J. S., U. S. Department of Agriculture, Washington, D. C.
Chesnut, V. K., Agricultural Experiment Station, Bozeman, Mont.
Church, C. G., U. S. Department of Agriculture, Washington, D. C.
Coates, C. E., State University, Baton Rouge, La.
Collins, A. T., The Colburn Company, Philadelphia, Pa.
Cook, F. C., U. S. Department of Agriculture, Washington, D. C.
Crampton, C. A., Treasury Department, Washington, D. C.
Cushman, A. S., U. S. Department of Agriculture, Washington, D. C.

Davidson, R. J., Agricultural Experiment Station, Blacksburg, Va.
Dennis, L. M., Cornell University, Ithaca, N. Y.
Donk, M. G., Assistant State Chemist, Tallahassee, Fla.
Doolittle, R. E., State Analyst, Dairy and Food Department, Lansing, Mich.
Dorset, M., U. S. Department of Agriculture, Washington, D. C.
Doyle, A. M., U. S. Department of Agriculture, Washington, D. C.
Drueding, C. F., Yocum Leather Trade Laboratory, Newark, N. J.
Dubois, W. L., U. S. Department of Agriculture, Washington, D. C.
Dyck, G. E., Official Chemist, Produce Exchange, New York, N. Y.

Emery, J. A., U. S. Department of Agriculture, Washington, D. C.
Ewell, E. E., German Kali Works, Atlanta, Ga.

Failyer, G. H., U. S. Department of Agriculture, Washington, D. C.
Fields, John, Agricultural Experiment Station, Stillwater, Okla.
Fischer, Richard, State Chemist, Madison, Wis.
Foster, A. B., Agricultural College, College Park, Md.
Frear, Wm., State College, Pennsylvania.
Friend, G. C., Union Abattoir Company, Baltimore, Md.

Gascoyne, W. J., 23 South street, Baltimore, Md.
Gibboney, James H., Agricultural Experiment Station, Blacksburg, Va.
Gore, Herbert C., U. S. Department of Agriculture, Washington, D. C.
Gough, T. R., Agricultural Experiment Station, College Park, Md.
Gray, J. P., Agricultural Experiment Station, College Park, Md.
Grindley, H. S., University of Illinois, Urbana, Ill.
Gudeman, Edward, 704 Rialto Building, Chicago, Ill.

Hand, W. F., Agricultural College, Miss.
Hardin, M. B., Agricultural Experiment Station, Clemson College, S. C.
Hartwell, B. L., Agricultural Experiment Station, Kingston, R. I.
Haskins, H. D., Hatch Experiment Station, Amherst, Mass.
Haywood, J. K., U. S. Department of Agriculture, Washington, D. C.
Herff, B. von, German Kali Works, New York, N. Y.
Hillebrand, W. F., U. S. Geological Survey, Washington, D. C.
Hiltner, R. S., Treasury Department, Washington, D. C.
Hird, J. D., Health Department, Washington, D. C.
Hollister, F. M., Agricultural Experiment Station, Burlington, Vt.
Hopkins, C. G., Agricultural Experiment Station, Urbana, Ill.
Houghton, H. W., U. S. Department of Agriculture, Washington, D. C.
Howard, B. J., U. S. Department of Agriculture, Washington, D. C.
Hurst, L. A., U. S. Department of Agriculture, Washington, D. C.
Huston, H. A., German Kali Works, St. Louis, Mo.

Jenkins, E. H., Agricultural Experiment Station, New Haven, Conn.
Jones, E. E., U. S. Department of Agriculture, Washington, D. C.

Kebler, Lyman F., U. S. Department of Agriculture, Washington, D. C.
Kerr, G. A., Acme Bark Extract Company, Damascus, Va.
Kerr, R. H., Agricultural Experiment Station, College Park, Md.
Kilgore, B. W., State Chemist, Raleigh, N. C.
Knight, C. P., U. S. Department of Agriculture, Washington, D. C.
Krug, W. H., A. Klipstein & Co., New York, N. Y.

Langworthy, C. F., U. S. Department of Agriculture, Washington, D. C.
Lawson, H. W., U. S. Department of Agriculture, Washington, D. C.
Leach, A. E., State Board of Health, Boston, Mass.

LeClerc, J. A., U. S. Department of Agriculture, Washington, D. C.
Lipman, J. G., Agricultural Experiment Station, New Brunswick, N. J.
Long, J. H., Northwestern University, Chicago, Ill.
Lord, E. C. E., U. S. Department of Agriculture, Washington, D. C.

McCarthy, E. R., Acme Bark Extract Company, Damascus, Va.
McDonnell, H. B., Agricultural Experiment Station, College Park, Md.
Magruder, E. W., Department of Agriculture, Richmond, Va.
Mason, G. F., D. J. Heinz Company, Pittsburg, Pa.
Mewborne, R. G., Kentucky Tobacco Product Company, Louisville, Ky.
Moore, C. C., U. S. Department of Agriculture, Washington, D. C.
Morse, F. W., Agricultural Experiment Station, Durham, N. H.
Mosbaugh, F. R., Huntsville and Bracebridge Tanning Company, Huntsville, Canada.
Mosser, T. J., State College, Pa.
Mudge, C. W., Agricultural Experiment Station, Geneva, N. Y.
Munson, L. S., U. S. Department of Agriculture, Washington, D. C.

Nichols, M. F., Leather Trades Laboratory, Grand Rapids, Mich.
Noyes, W. A., Bureau of Standards, Washington, D. C.

Page, L. W., U. S. Department of Agriculture, Washington, D. C.
Patrick, G. E., U. S. Department of Agriculture, Washington, D. C.
Patterson, H. J., Agricultural Experiment Station, College Park, Md.
Penny, C. L., Agricultural Experiment Station, Newark, Del.
Perkins, W. R., Agricultural Experiment Station, Agricultural College, Miss.
Price, H. L., Virginia Polytechnic Institute, Blacksburg, Va.
Price, T. M., U. S. Department of Agriculture, Washington, D. C.
Putnam, Alice, Anacostia, D. C.

Reed, H. C., Stamford Manufacturing Company, Stamford, Conn.
Richardson, Clifford, New York Testing Laboratory, Long Island City, N. Y.
Richtmann, W. O., 1615 Florida avenue, Washington, D. C.
Robb, J. B., Agricultural Experiment Station, College Park, Md.
Robison, F. W., Agricultural Experiment Station, Agricultural College, Mich.
Ross, B. B., State Chemist, Auburn, Ala.
Runyan, E. G., U. S. Inspector Gas and Meters, Washington, D. C.

Sawyer, H. E., 163 C street, South Boston, Mass.
Schreiver, Oswald, U. S. Department of Agriculture, Washington, D. C.
Scovell, M. A., Agricultural Experiment Station, Lexington, Ky.
Seidell, Atherton, U. S. Department of Agriculture, Washington, D. C.
Shutt, F. T., Central Experimental Farm, Ottawa, Canada.
Simons, F. D., Treasury Department, Washington, D. C.
Smith, P. H., Hatch Experiment Station, Amherst, Mass.
Smith, B. H., U. S. Department of Agriculture, Washington, D. C.
Smith, J. G., U. S. Department of Agriculture, Washington, D. C.
Snyder, Harry, University of Minnesota, St. Anthony Park, Minn.
Stilwell, A. J., Leather Trade Counsel, Dubois, Pa.
Steinharter, J. J., Shoe and Leather Reporter, Philadelphia, Pa.
Stokes, H. N., Bureau of Standards, Washington, D. C.
Stone, W. E., Purdue University, Lafayette, Ind.
Straughn, M. N., Agricultural Experiment Station, College Park, Md.
Street, J. P., Agricultural Experiment Station, New Brunswick, N. J.

Talbot, H. P., Massachusetts Institute of Technology, Boston, Mass.
Teas, W. H., Elk and Penn Tanning Companies, Ridgway, Pa.
Tolman, L. M., U. S. Department of Agriculture, Washington, D. C.
Trescot, T. C., U. S. Department of Agriculture, Washington, D. C.

Van Dyke, J. H., U. S. Department of Agriculture, Washington, D. C.
Van Slyke, L. L., Agricultural Experiment Station, Geneva, N. Y.
Veitch, F. P., U. S. Department of Agriculture, Washington, D. C.
Voorhees, E. B., Agricultural Experiment Station, Washington, D. C.

Warner, H. J., U. S. Department of Agriculture, Washington, D. C.
Weber, H. A., State University, Columbus, Ohio.
Weber, F. C., U. S. Department of Agriculture, Washington, D. C.
Wharton, W. R., Agricultural College, College Park, Md.
Wheeler, H. J., Agricultural Experiment Station, Kingston, R. I.
White, H. C., Athens, Ga.
Wiley, H. W., U. S. Department of Agriculture, Washington, D. C.
Wiley, S. W., American Agricultural Chemical Company, Baltimore, Md.
Williams, C. B., Department of Agriculture, Raleigh, N. C.
Wilson, H. T., Buena Vista Extract Company, Buena Vista, Va.
Winton, A. L., Agricultural Experiment Station, New Haven, Conn.
Withers, W. A., Agricultural Experiment Station, Raleigh, N. C.
Woll, F. W., Agricultural Experiment Station, Madison, Wis.
Woods, C. D., Agricultural Experiment Station, Orono, Me.

The order of business was as follows:

ORDER OF BUSINESS.

The president's address.
Appointment of committees.
Reports of the referees, in the following order:

1. Report on nitrogen.
2. Report on potash.
3. Report on phosphoric acid.
4. Report on soils.
5. Report on ash.
6. Report on foods and feeding stuffs.
7. Report on food adulteration.
8. Report on dairy products.
9. Report on sugar.
10. Report on tannin.
11. Report on insecticides.
12. Reports of special committees (food standards, fertilizer legislation).
13. Reports of committees A, B, and C, on recommendations of referees.

PRESIDENT'S ADDRESS

Gentlemen of the Association of Official Agricultural Chemists: At the opening of this, the twentieth annual convention of our association, it will not be amiss for us to consider a few points in connection with the work which first brought this association into existence. The object of this work was to secure uniformity of methods for the determination of the valuable ingredients in commercial fertilizers. The scope of the association was broadened later, so that, as the constitution now reads, its objects are: "To secure uniformity and accuracy in the methods, results, and modes of statements of analysis of fertilizers, soils, cattle foods, dairy products,

and other materials connected with agricultural industry; (2) to afford opportunity for the discussion of matters of interest to agricultural chemists."

How well the first part of this work, in regard to uniformity and accuracy of methods, has been done is fully attested by our official methods, which are now universally acknowledged as the proper legal methods for the determination of the constituents of commercial fertilizers. But with the advancement of the work in this direction other problems have appeared which are now occupying the attention of our referees, such as the determination of iron and aluminum in phosphates, and of phosphoric acid in basic slag. These points will in time, I have no doubt, be properly provided for in our scheme of analysis.

It is not alone the question of chemical analysis to which I wish to call attention, but also that of the establishment of uniform laws and proper standards for the inspection of commercial fertilizers, a matter intimately connected with and dependent upon chemical work. This is a vital question in connection with our duty as official chemists, and a more troublesome one perhaps could not be brought up, since so many varied conditions must be considered.

Many of our States now have laws for the regulation of the sale of commercial fertilizers, but between the 23 whose laws I have examined there is no mutual agreement either as to requirements or form of statement of composition. When we consider the immense sums of money annually invested in these materials and the large number of people who purchase them, and how few of this number have a clear understanding of the character of fertilizing materials, it behooves this association to exert all its influence to make these laws conform to some definite standard, and to aid as far as possible in having plain, simple, and intelligible statements made in regard to the constituents contained therein. At this point it might be well to mention the fact, which is well known to most of the members of the association, that our National Government has recognized this body as the proper one to aid in the formation of standards for pure foods. It would, therefore, seem reasonable to assume that the several States interested in the question of fertilizer laws would be very glad to make use of a similar standard of requirements on this subject prepared for their use by this association. These standards have been prepared by our committee on uniform fertilizer legislation, and their report was adopted by the association five years ago. It has also been adopted by the American Association of Agricultural Colleges and Experiment Stations. Although five years have elapsed since this report was published, the State laws are still far from uniform, and some passed within the last year, although the question of uniform standards was brought up, still are framed according to the old ideas, although some of the new requirements were adopted. Why is this? It certainly is not due to the lack of importance of the subject. It is true the conditions existing in the various States are widely different; yet I do not think this is the true reason. Let us look for another. As in all the work of the association individual effort is very important—the results falling short of what they should be when it becomes weak—and in this case I think individual effort is lacking. Our committee has done all in its power to have this work brought to the attention of the authorities in those States where requests for information were made, and one or two of our members have exerted their influence with good effect. But every member of this body should use his influence in his State to have the question of the modification of existing laws, or the enactment of new ones conforming to the standards adopted by this association, brought before the proper authorities at the proper time.

I suppose most of the members are familiar with the standards that have been adopted on this subject, so I will not quote all of them; but I would like to make a comparison between some of the requirements and those found in a number of our State laws. The question of nitrogen is one of the most important; so let us consider it. Our standard requires "total nitrogen guaranteed in all cases, and nitrogen in

the form of nitrates or ammonium salts guaranteed separately if the manufacturer desires credit therefor." This latter point is a very important one, and the constant complaint of the manufacturer in many of our States is that he gets no credit for nitrogen as nitrate. Of the 23 State laws examined 8 only require the per cent of available nitrogen, and that expressed as total nitrogen only. Nine prohibit the use of leather in any form, and 3 leather, wool waste, hair horn, and other inert nitrogenous material, unless a printed explicit statement is attached to each package, or such a statement made to the commissioner or board of agriculture. One prohibits the use of leather in any form, and 2 prohibit leather, hair horn, and hoof meal, etc. Eleven (about one-half) require a statement of per cent of nitrogen; 7 require per cent of nitrogen or its equivalent of ammonia; and 3 require statement of per cent as ammonia. No State law examined requires the separation of the different forms of nitrogen. As nitrogen is the most expensive constituent of commercial fertilizers, the greatest safeguards as to its condition and use should be placed about it, for the sake of both purchaser and honest manufacturer. But many of our State laws leave it so unguarded that it may be furnished in forms of practically no value for fertilizing purposes, while the purchaser has to pay as if it were of the highest value. In stating percentages only minimum amounts should be given, but of the 23 laws examined, only 6, or about one-fourth, called for the minimum alone.

I would like, therefore, to call the attention of every member of this association who is connected in any way with official fertilizer control in his own State to the necessity of attending to this important part of his work. It is the duty of the official chemist to prevent adulteration and fraud by every legitimate method available. I should like also to call attention to a very important point, and our committee might take it under consideration, which should be made a part of the standards on this subject. This is the limit of error or deficiency which should be allowed on the various constituents in commercial fertilizers and fertilizing materials. Cattle foods might be included also. There is a very much wider difference on this point in our different State laws than on any other. Of the 23 examined, 7 have vague enactments, such as "* * * shall lack materially any valuable ingredient." Such a statement is very indefinite, for who is to judge where "immaterial lack" ends and "material" begins. Five have a limit based on percentage composition, 3 on commercial valuation. Of those based on percentage composition, 2 allow a limit of 1 per cent. This is a rather liberal allowance, and unscrupulous manufacturers could make money by guaranteeing and charging for, say, 2 per cent of the nitrogen and only giving 1 per cent. One law allows one-fourth of 1 per cent on all constituents, and one allows one-half of 1 per cent on 2 constituents and one-third on the other. Of those based on commercial valuation one reads: "When it is essentially below guaranteed commercial value." Two allow a deficiency of 3 per cent below guaranteed value, and one 10 per cent below guaranteed total commercial value. No doubt it would be a great relief to all parties concerned, and especially to the chemist, to have some fixed and definite standard. Of the various methods now used for determining deficiency, the one based on percentage composition is undoubtedly the best, and would, in my opinion, be the most agreeable to all parties concerned.

The question of determining the different forms of nitrogen in fertilizers has been raised by our resolutions, and yet with one or two exceptions the chemist does not determine the amounts of nitrogen as nitrate or ammonia salts separately, but simply states his results as total nitrogen. In some cases the total is given as ammonia. The purchaser is therefore left completely ignorant of the value of the material. The following resolution was adopted by the association in 1900: "It is desirable that the form of nitrogen should be determined and reported on in analysis of fertilizers." That this has not been done certainly may be the fault of our State laws in not urging it, but I think it is also to some extent the fault of the individual chemist who is making the analysis. The law does not say that he shall not make these sep-

arate determinations, therefore, in the interest of the people, both manufacturers and consumers, he should do this for their information; and by so doing he would in time create such a demand in his State for this information that the people would have it embodied in the law. In order to have laws of any sort we must educate public sentiment and create a demand for the law before we get it, and this is true in the case of our fertilizer work. If we want uniform fertilizer laws we must see to it that our people, and especially those directly concerned, should have all the information necessary to a correct understanding of the merits of the question. This can be done more effectually by the official chemists in their various reports, bulletins, and lectures at farmers' institutes than in any other way, because the people have confidence in us, and know that our aim is for the greatest good of the greatest number, and that we have no special ax to grind.

There should also be a movement to prohibit the misnaming of fertilizers; such as the term guano for an artificial mixture of phosphoric acid, nitrogen, and potash, and the use of the term dissolved bone, for dissolved phosphate rock. I know the association has no power in this matter save that of influence, but its strength as a scientific, conscientious, and conservative body, having the best interests of the people at heart, is recognized throughout the country, and it is therefore expected to take the initiative in all lines of work with which the official chemist has to deal.

In this connection how prophetic sounds a statement made in 1889 by our lamented associate, Dr. J. A. Myers, in his address as president of the association:

> If this association continues to maintain its proper attitude and high sense of the responsibilities resting upon it, we may safely say that the time is not far distant when its deliberations will virtually become the law regulating the sale of nearly every agricultural and food product offered in our cities.

The growth of the association in amount of work and in numbers has kept pace with the importance of its mission. In 1893 there were seven reporters on the various subjects then under consideration, and these are still before the association, but the subjects have been enlarged and the number increased until now we have eleven referees and thirty-four associates, and still the work and responsibilities multiply. We are to have at this meeting reports by the various associate referees on food adulteration, a comparatively new subject undertaken by the association only a year or two ago. This is the beginning of our work toward the adoption of uniform methods of analysis and standards for food products, and, as stated before, the results of this work will be used in the execution of a national pure-food law when passed.

But with all the increased amount of work that is being done there is still a great deal more ahead of us. If we expect to live and grow we must enlarge our work and our responsibilities; we must not stand still and live on what has been done. More investigation work is needed, and each and every member of the association should feel it his duty to do something each year, either as an individual worker on some special line or in assisting the referees in their efforts to improve and add to our present methods of analysis.

In looking over the list of members who have attended our last two meetings, the eighteenth and nineteenth annual conventions, we find there were present at the former meeting 118 and at the latter 100 members. How many of these took an active part in the work of assisting our referees? The largest number working on any one subject is about twelve, and on the subjects of soil and insecticides only about four or five. This looks like lack of interest in the referees' work. Of course, it is not expected that everyone will do work for all the referees, but it is expected that everyone will do some work each year on some one or more of the subjects under consideration by the association.

Another point of interest in connection with our last two meetings is the small amount of time given to discussion. The opportunity for discussing the various lines of work on the floor of the convention is very important, adding greatly to a better

understanding of the questions at issue, and increasing materially the attractiveness of our meetings. I think too much of the discussion is reserved for the various committees who have the several subjects in charge. I have no doubt they would be very glad to be relieved of some of the discussion, and have the greater part presented before the whole convention. The committee could then take the question under advisement, and if any additional facts are needed before submitting the report to the whole body, they could be called for. The convention, having heard the discussion of a question, would be better able to vote intelligently on the committee's report. It would not then be necessary, as is often the case now, for members continually to interrupt the chairman making the report by asking questions.

I would, therefore, plead for more discussion on the floor of the convention; and in order to give time for this would suggest that each referee in making his report be as clear and concise as possible, only reading such figures as are necessary to a clear understanding of the questions under consideration. It is not necessary to read the results obtained by the different analysts, especially when in many cases the members have the table in question before them.

I would also recommend to the consideration of the association the question of the adoption of a uniform method of statement of results on all the subjects which we have under consideration. It is hardly necessary for me to say how this would benefit us in our work. We all know how much difficulty we have when we are looking up results for comparison or getting up a compilation. We find results given in so many different ways and forms that it takes considerable work to figure them out, and, indeed, many results can never be used because sufficient data is not at hand to make calculations. I would also recommend that a change be made in the temperature at which our measuring apparatus is graduated. The temperature at present used, in nearly all cases, is 15.5° C. This is very much below room temperature, and often requires the cooling of solutions—a troublesome process, consuming considerable time. It seems to me that it would be of decided value to us if a temperature were adopted which would more nearly correspond to the temperature of our laboratories, which is usually about 20° C.

In conclusion I wish to renew a recommendation that was made last year by the last president, but which was not acted upon; that is to have the association consider the advisability of publishing Battle and Dancy's chemical conversion tables for the use and convenience of our members. These tables are now out of print, and I understand that the authors, if given proper credit for the same, are perfectly willing for the association to make such use of them as it may desire.

APPOINTMENT OF COMMITTEES.

The PRESIDENT. The appointment of committees is in order, and I will make the following appointments:

Committee to wait on the Secretary and Assistant Secretary of Agriculture: Messrs. Winton, Street, and Penny, Doctor Munroe, dean of Columbian University, and the president of the association.

Committee on resolutions: Messrs. Kilgore, H. B. McDonnell, and Jones.

Committee on nominations: Messrs. Wheeler, Frear, and Ross.

Committee on amendments to the constitution: Messrs. Patterson, Hills, and Magruder.

If there is no objection I will appoint the same number of committees on recommendations of referees as were named last year.

Committee A (phosphoric acid, potash, nitrogen, soils, ash, and insecticides): Messrs. Hartwell, Ross, Hopkins,[a] and Kilgore.

Committee B (dairy products, foods and feeding stuffs, sugar, and tannin): Messrs. Patrick, Withers, Van Slyke, and Kerr.

Committee C (food adulteration): Messrs. Leach, Munson, Penny, and Eaton.[b]

The PRESIDENT (continuing). The report of the referee on food adulteration is in order.

REPORT ON FOOD ADULTERATION.

By W. D. BIGELOW, *Referee.*

Heretofore the work on food adulteration has been largely done in committee, as you remember, and the action of the committee only has been reported to the association as a whole. It was necessary to do this because of the great amount of material that had to be gone over every year, much of which was attended to by correspondence, and even a small committee required two days at least, and sometimes more, to cover the ground. This year it was considered that we had reached the point where the work could be taken up in the association, and a number of reports have been prepared by subreferees. Unfortunately, in many cases the subreferees have not been able to present a report, owing to the fact that it was impossible to secure collaboration over the entire field. No report has been received for saccharine products—wine, beer, vinegar, baking powder, cereal products, infant foods, vegetables, tea, and coffee. Considerable work has been undertaken on cereal products, but, with the exception of one paper which will be presented here, nothing has been completed, ready to report. One paper by Mr. Chamberlain, relating to the separation of the nitrogenous constituents of flours, while prepared in connection with the work on cereal products, would be more properly presented in connection with the report on the separation of nitrogenous bodies, the study of the vegetable, meat, and milk and cheese proteids having been assigned to a special referee by the association two years ago. Also, work that was taken up by the referee on food adulteration a couple of years ago, and has been continued this year both by Mr. Grindley, on meat, and in the food laboratory of the Bureau of Chemistry on meat extracts, is to be reported at this meeting, but will probably come under the report on the separation of nitrogenous bodies also, although the work was begun in connection with the food methods, and has been largely conducted under that heading until the last year.

I would suggest that the various associate referees now take up the questions that have been assigned to them in their order. In regard to the subject of colors, which comes first on the list, I have a provisional report to submit from Mr. Berry, who has one of the most difficult subjects and has made a good beginning, but was unable to attend the meeting.

REPORT ON COLORS.

By W. G. BERRY, *Associate referee.*

Owing to the large amount of work necessary for the preparation of a complete and systematic résumé of the subject of colors in connection with food stuffs, and the failure of collaboration, I am unable to present a finished report on the subject, and desire therefore to defer the final report until next year. The following synopsis of

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[a] Mr. Grindley was later appointed in the place of Mr. Hopkins.

[b] Mr. Hird was later appointed in the place of Mr. Eaton.

the subject, with extracts from the incompleted work in hand, will give some idea of the manner in which the subject will be treated.

It is suggested that the members of the association be invited to do what original work their time may permit of, on the points suggested below, and communicate their results to the associate referee during the ensuing year, for incorporation in the final report.

(1) Solubility of the coal tar and vegetable dyes in various solvents (ether, acetic ether, petroleum ether, methyl and ethyl alcohols, acetone, etc.) arranged according to their solubility—as easily soluble, difficultly soluble, and insoluble.

(2) Extractive values of the various solvents for dyes in neutral, acid, and alkaline solutions.

(3) Characteristics of the coloring matters as contained in fresh fruits, vegetables, wines, etc., with reagents and solvents, and dyeing properties.

(4) Testing such new schemes as may appear in the various chemical journals, or have appeared during the last few years.

Synopsis of Report on Coloring Matters in Food Stuffs.

For convenience in handling, the subject will be treated in a systematic arrangement under the following headings, viz:

I. Remarks in general on coloring matters liable to be present in food stuffs.
II. Colors to be considered.
  (*a*) Coal-tar colors, with their composition, commercial designations, and synonyms.
  (*b*) Vegetable colors; their botanical origin, common names, etc.
  (*c*) Mineral colors and pigments.
  (*d*) Organic lakes.
III. Harmless and harmful colors and dyes, so far as known.
IV. Commercial coloring compounds.
V. Grouping of food stuffs into general classes for examination, as follows:
  1. Butter, oils, milk, cheese, etc.
  2. Nitrogenous foods.
    (*a*) Flesh foods.
    (*b*) Canned vegetables.
    (*c*) Beer, malt liquors, etc.
  3. Starch foods (noodles, macaroni, etc.).
  4. Vinegar.
  5. Confectionery.
  6. Wines and spirituous liquors, fruit sirups, etc.
  7. Brandy, liqueurs, etc.
  8. Tea, coffee, spices, etc.
  9. Medicinal preparations.
  10. Cosmetics, soaps, etc.
  11. Miscellaneous material (tags, paper linings, etc.).

  Each class will be treated under the following heads:
  (*a*) Natural or artificial coloring.
  (*b*) Mineral or organic coloring.
  (*c*) General methods in detail for detecting the organic coloring matter, and, if possible, isolation of same for identification by tabular schemes.
  (*d*) Special tests for colors.
VI. Each of the eleven classes in detail under heads as given in V.
VII. Original work in behavior of coloring matters with solvents and reagents.
VIII. Tabular schemes for the identification of the isolated coloring matters.
IX. Addenda (what colors allowed, laws, etc.).

I.—REMARKS, ETC.

The field of color chemistry is very large, and the chemist in general analytical practice often neglects it owing to the vast amount of time and patience required for an intelligent understanding of the subject. Much good work has been done toward establishing methods for the recognition of coloring matters in food stuffs, but with

the data at present available for such work the individual operator will find himself called upon to exercise his own judgment and apply his own experience to the subject-matter in hand.

The introduction of coal-tar dyes into the field of food stuffs complicates enormously the scope of the work to be covered in an examination of artificial coloring. Were it possible to limit the examination to a certain number of colors, the work would be materially simplified, but no sooner has the analyst perfected color schemes and tabulated reactions for the identification of such colors when new ones are found, and render previous schemes to a certain extent unreliable and necessarily subject to alteration.

Hence the most that can be hoped for is to establish general methods for determining whether artificial coloring has been resorted to, and if so, whether the coloring matter is of mineral, vegetable, or coal-tar origin. This fact having been established, special methods must be applied for the separation and identification of the individual coloring matters, often complicated by the presence of mixtures of several coal-tar colors, rendering color reactions useless until each of the separate colors has been isolated. The synopsis given is intended to be an outline of methods for the examination of food stuffs, to be elaborated and extended in the future.

The food chemist may be called upon for an examination of the coloring matters and commercial preparations used for coloring foods, and for the examination of foods for the presence of artificial colorings, identification of the added color, and the presence of poisonous metals due to the latter. He may also be called upon for an opinion as to the toxic or nontoxic character of the coloring matter, which not only requires an examination for poisonous metals present, as lakes and pigments, but in addition the determination of the presence of poisonous metals due to impurities, the identification of the color or colors, and a knowledge of the toxic effects of the pure color per se.

An examination, therefore, of the colors and preparations employed involves the identification of the heavy metals present, the nature of the color entering into the lake, whether vegetable or of coal-tar origin, its identification, and, in the case of mixed colors, their separation and identification, and, lastly, the presence of poisonous metals as impurities in the coal-tar dyes, or their presence as inherent constituents of the colors themselves.

Many of the colors contain metallic constituents, as in the triphenylmethane derivatives, especially the greens, such as malachite green, iodin green, ethyl green, victoria green, etc., which contain a double chlorid of zinc in combination with the organic matter. Some of the acridines and thiazines also contain zinc and naphthol green B contains iron.

In the preparation of the coal-tar dyes many become contaminated with arsenic, copper, zinc, tin, lead, etc., from the use of these metals and their salts during the process of manufacture. Sulphuric acid being used in some stage of the process of manufacture of nearly all the dyes, introduces the danger of arsenic being present. Hence all dyes used for artificial coloring, which in themselves are harmless, may become injurious from the presence of these poisonous ingredients, unless highly purified.

While alum lakes of the vegetable colors are in general harmless, the presence of lakes of tin, antimony, etc., should be carefully guarded against.

The vegetable colors should be examined for the presence of coal-tar dyes of a dangerous nature, and for other vegetable colors and substances and inert mineral matter added for cheapening.

The examination of the food stuffs themselves necessitates determining whether the coloring is due to natural or artificial means, and, if the latter, the color or colors must be separated and an examination of the material made for heavy metals.

In regard to the toxic effects of the purified coal-tar dyes, the chemist must rely

upon the experiments and conclusions of investigators in this line of work, and should hesitate to express an opinion on a new color without a physiological test upon living organisms. The experiments of König, Weyl, and others should be consulted on this subject.

The methods of separating the colors from the material to be examined by the use of solvents involves the separation of the natural colors at the same time, and these must first be isolated before identification tests can be applied. This fact naturally necessitates a very comprehensive knowledge of the color reactions and the behavior of the natural colors with reagents. Complete data on this subject are as yet wanting.

The coal-tar dyes may, in general, be most satisfactorily separated by the double-dyeing method, with or without previous extraction with solvents, as the case may be, and in some cases may be identified directly by tests on the dyed fabrics, or more accurately by being removed from the fabric and subjected to purification. Great care and judgment must be exercised in applying the color reactions and arriving at a conclusion therefrom, as the presence of more than one dye, or some organic impurity, will lead to erroneous results. Several means of identification should be tried and a conclusion drawn only when the analyst is perfectly satisfied of the identity of the dye.

Many of the results obtained in the determination of a dye have been rendered ambiguous, owing to confusion of names applied to the same dye, and the analyst should always make a point of identifying any particular dye by giving the name of the original manufacturers, so that its composition may be known. As, for instance, Orange G might be either of the following, in default of a distinguishing mark, viz:

Orange G (C. J.). Anilin azo β naphthol.

Orange G (B). Anilin azo 2 naphthol, 6.8 disulphoacid sodium salt.

Orange G (H). Sulphanilic acid azo β naphthol sodium salt.

As a guide to the food analyst, a compilation is given below of the vegetable and animal colors, lakes, and pigments, and the coal-tar dyes, with their synonyms, which he may be called upon to examine. Many of the old vegetable colors have, of course, been discarded for the coal-tar dyes now so universally employed.

V.—FOOD STUFFS, ETC.

1. Butter, oils, milk, cheese, etc.

The natural coloring matter of milk is lactochrome, which may be precipitated from milk whey by nitrate of mercury in bright, red-orange, resin-like masses, softening at 100° C., freely soluble in water and hot alcohol, separating from the latter on cooling (Blyth). Cholesterin occurring in milk is soluble in hot alcohol, ether, carbon disulphid, and chloroform, and when treated with five parts sulphuric acid to 1 part water it is colored red, then violet (Moleschott's test); and with 1 part sulphuric acid to 1 part chloroform, is colored blood red, violet, or purple (Salkowski's test).

The coloring matter of some plants when eaten imparts color to milk of cows, as follows: Marsh marigold, yellow; dyer's madder, red; and true forgetmenot and knotweed, blue.

Certain bacteria develop a blue color in milk, which turns cherry red on addition of caustic alkalis, and returns blue on adding acids.

Added vegetable colors present may be derived from safflowers, carrot juice, marigold flowers, celandine, buttercups, turmeric, annatto, etc.

Coal-tar colors present may be spirit yellow R, nitrosamin red, aurantia, methyl orange, orange IV, phosphine, picric acid, Martins yellow, Mikado yellow, Mikado gold yellow, naphthol yellow S, amidoazobenzol, butter yellow (benzine-azo-dimethyl-anilin), naphthol yellow RS, Victoria yellow, coralline yellow, anilin yellow (amido-azo-benzol-hydrochlorid), acid yellow, diazo-benzine, etc.

Color preparations are carottine, a solution apparently of 1 part annatto in 4 parts of oil, turmeric taking the place sometimes of the annatto; orantia, a mixture of annatto, sodium carbonate, and water, etc.[a]

(a) Butter, fats, oils, etc. (fresh).

Added coal-tar colors will be present, with few exceptions, in the form of nonsulphonated bases, and will be indicated by the test of Bujard and Baier.[b] From 2 to 3 grams of fat are dissolved in 5 cc of ether and shaken with 5 cc of concentrated hydrochloric acid (1.125). If coal-tar colors are present the solution is colored decidedly red. "Butter yellow," however, colors hydrochloric acid (1.19) only slightly or not at all.

J. Vandriken[c] states that pure butter is completely decolorized by amyl nitrite. Proceed as follows:

1. *Amyl nitrite (acid).*—To 2 cc of filtered butter is added an equal volume of ether in a test tube. To this 6 to 10 drops of amyl nitrite are added and then shaken. Pure butter is decolorized at once. If unfiltered butter is used more reagent must be employed, and usually slightly warmed.

2. *Nitrous ether.*—To 2 cc filtered butter add 2 cc of ether, then 25 to 30 drops of nitrous ether, and shake vigorously. The decolorization takes place more slowly than with amyl nitrite. Carotin is not decolorized, saffron is only slightly altered, turmeric is not decolorized, and annatto is not decolorized.

Leed's method is given in detail in the Analyst.[d] In using this method, if the butter has become rancid the acid water from the petroleum-ether extract must be tested for reduced coal-tar dyes as given under milk.

Special tests for saffron, turmeric, marigold, and annatto are given by Martin;[e] for carotin, by Moore;[f] and for caramel, by Leach.[g] These methods are given in detail, and in various authorities special tests may be found for saffron, turmeric, carotin, and rocou.

(b) Milk (fresh).

Leach's method is given in detail in the Journal of the American Chemical Society.[h]

(c) Milk (sour).

Blyth's method is given in detail in the Analyst.[i] This method is important for detecting the presence of coal-tar dyes which have been reduced to colorless compounds due to fermentation.

(d) Cheese.

Extraction with hot alcohol for coal-tar dyes. Shake with petroleum ether for nonsulphonated and vegetable colors and proceed as with butter and milk.

(e) Schemes.

Some special color reactions tabulated for vegetable and coal-tar dyes occurring in the above class of foods.

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[a] Allen, Com. Org. Anal. 1889, **3**: 356.
[b] Hilfsbuch für Nahrungsmittelchemiker, p. 144.
[c] Chem. Ztg. Rep. 1901, p. 106.
[d] Vol. 13, p. 150.
[e] Analyst, **12**: 70.
[f] Analyst, **11**: 163.
[g] J. Amer. Chem. Soc., **20**: 207.
[h] J. Amer. Chem. Soc., 1900, p. 207.
[i] Analyst, 1902, p. 146.

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The PRESIDENT. If there is no discussion the report of the associate referee on distilled spirits will be received.

REPORT ON DISTILLED SPIRITS.

By C. A. CRAMPTON, *Associate referee.*

Under date of June 10, 1903, the associate referee sent the following circular letter to six chemists whose names had been suggested as likely to undertake work on liquors:

DEAR SIR: Concerning the collaborative work for the Association of Official Agricultural Chemists, upon distilled spirits, in which you have expressed a willingness to take part, you are informed as follows:

The most important inquiries to be answered in judging of the character of a sample of distilled spirits are:

(1) Does it contain ingredients deleterious to health? This is determined by the tests for furfurol, and adventitious, harmful additions, and, to some extent, by the estimation of fusel oil.

(2) Is it genuine or factitious?—i. e., does it owe its flavor and color to a long period of aging in wooden vesssels or has it been made from neutral or cologne spirits by adding coloring matter, or artificial flavoring, or both. The determination of the amount and character of the solid matter present usually affords the answer to this question, supplemented by tests for artificial coloring matter. By far the greater part of the spirits consumed in this country is of the latter class, which is, doubtless, as healthful as the former class, but a much cheaper product. The difficulties of discriminating between the two are, of course, greatly increased in the case of a mixture.

On account of the small number of chemists who have offered to collaborate, I have not thought it advisable to send out identical samples. Samples of genuine spirits can be readily obtained in the shape of goods "bottled in bond," sold under a strip stamp which covers the cork of the bottle, and which gives the age of the spirits. Samples of factitious spirits can he made up by the analyst himself, or obtained in the shape of cheap rectified spirits. Genuine spirits are not all necessarily covered by a warehouse stamp; some very expensive whiskies are made by blending different varieties, which can not be done in bond.

The nature and number of the estimations to be made I will also leave to the judgment of the individual operator. I would, however, recommend the careful analysis of a few samples, including all the usual determinations, rather than the application of a few estimations to a large number of samples. The determination of extract or total solid matter in a large series of samples of known purity would, however, be of value in establishing a standard, and I should like to have such results.

In addition to the determinations described on pages 96 to 98, Bulletin No. 65, Bureau of Chemistry, U. S. Department of Agriculture, Provisional Methods, etc., I would recommend the determination of the copper oxid reducing power and its expression as a ratio of the per cent of extract, also the solubility in boiling water of the extract (a method recently published by Lasche and Kiesslich in the Brewer-Distiller, 1903, 1), expressed in the same way. As an alternative method under "11.—Determination of coloring matter" besides the paraldehyde and ether solubility tests, I would be glad to have the fuller's earth absorption test subjected to examination (Crampton and Simons, Journal of the American Chemical Society, 1899, 21: 355), and will furnish a sufficient quantity of the fuller's earth used in this laboratory for that purpose, if notified.

Please do not hesitate to ask for any additional assistance or suggestions concerning the work, if you so desire; the small number of chemists participating renders the work of correspondence comparatively light.

Your report, containing results and suggestions, should be sent in not later than September 1.

Only two reports were received, namely, those of Messrs. Pilhashy and Sawyer. Both contain valuable analytical data and suggestions, and are given in full.

REPORT OF H. E. SAWYER, BOSTON, MASS.

A tabular statement of the results obtained in the collaborative association work on distilled spirits is appended. Report is made at this time on 15 samples—2 of "French" spirit, at proof strength, such as is used here for splitting genuine goods;

4 of our new rum, taken at time of drawing; 4 of our old rum, taken from original packages; and 5 of goods which were supplied by retail dealers in Boston upon demand for old pure rum for medicinal use. As all of the official analytical methods could not be tested, those which seemed likely to be most significant were tried, leaving those which promised less for later examination. The extract and fusel-oil determinations, not reported here, are under way. The methods tested are those for acids (free and combined), aldehydes, furfurol, and coloring matter. Concerning them the following comments and suggestions are made:

Acids, free and combined.

Occasionally samples of new rum contain more or less carbonic acid, which will raise the apparent percentage of acetic acid when decinormal soda and phenolphthalein are used for titration. Also, it is very difficult, when working close to a fermenting room, to protect solutions against the carbonic acid of the atmosphere. On this account baric hydrate was tried in place of a caustic alkali. So far as could be determined with a few analyses, it works very well. One's attention is called immediately to the presence of CO_2; and, in addition, certain differences are perceptible between the deportment of genuine and colored goods. Cold saponification is also being tried in place of boiling under reverse cooler. With a large number of samples, and limited desk room, it is believed that it will prove to be advantageous.

Aldehydes.

So far the writer has been unable to prepare aldehyde-free alcohol, and on this account colorations have been compared with an arbitrary scale made up by progressive dilutions of a fuchsin-methyl violet mixture. Accordingly the aldehyde determinations given can not be compared with those of other workers, but are included in the report chiefly because they show a distinct increase in the strength of the color reaction in old rum. Four samples only were scaled—2 new and 2 old; but more than a dozen other samples were examined comparatively, and without exception the older rum gives the stronger reaction.

The Simon trimethylamine-sodium, nitroprussid test for acetaldehyde will not work in rum, although in a proof spirit made from specially pure alcohol it was obtained.

Furfurol.

Furfurol, like the aldehydes in general, seems to increase during storage, but it is not certain whether this is real or only apparent. I am inclined to believe in its reality, on account of the abundant production of the aldehyde in question during the charring of oak wood. It is intended to compare some uncharred packages with goods of the same age in charred barrels.

But it may be that there is an apparent difference, due to the presence in new rum of some material which, in part, obscures the furfurol reaction. On adding colorless c. p. anilin to colorless new rum a pronounced greenish-yellow coloration is at once developed. The same thing happens, though to a less extent, with some samples of commercial "proof spirit," such as the rectifiers use for splitting. The subsequent addition of acetic acid gives rise to the development of a pinkish coloration which is hard to estimate quantitatively, since it is obscured by the yellow, and in addition is very fugitive. In no case was the pink component of a new rum so determined, over 5 mg of furfurol per proof liter. If, however, the rum be redistilled before testing, the yellow tint is cut down materially, the pink component is at least doubled, and the red coloration is rendered far more permanent. (See samples 5 and 6.)

Coloring matters.

Both of the association methods for coloring matter were tried, using fuller's earth. With an earth which has been tested against several samples of known composition and with a good tintometer it is believed that a very close determination of added caramel, using the two methods in conjunction, may be made.

In connection with the matter of color, it is suggested that the extract of genuine aged spirits contains a good deal of tannin, whereas that of factitious liquors is not likely to. A test with ferric salts may be of value, and some work is being done on this point.

Fusel oil.

The official method has been inapplicable in my laboratory [Boston] during the summer on account of the temperature requirements. Therefore, the Allen-

Marquardt and Beckmann methods are being used, and ultimately it is hoped to compare the better of them with the official method.

The work, so far, has led to the conclusion that the acid and ester determinations are very significant. Their indications regarding samples 11–15 are fully in accord with the smell and taste of the liquors.

Results obtained by H. E. Sawyer.

No.	Sample.	Proof.	Acid.	Esters.	Aldehyde.	Furfurol.	Color left after fuller's earth treatment.	Color removed by ether.
			Gram per 100 cc.	*Gram per 100 cc.*	*Degrees.*	*Gram per 100 cc.*	*Per cent.*	*Per cent.*
1	Spirit	100.0	0.004	0.004	10	[a] 0.0002		
2	....do	100.0	0.001	0.0004	10	[a] Trace.		
3	New rum	100.0	0.025	0.101		[a] 0.0005		
4	....do	100.0	0.037	0.127		[a] 0.0005		
5	....do	112.4	0.037	0.113	55	[a] 0.0005 [b] 0.0010		
6	....do	99.8	[c] 0.076	0.084	55	[a] 0.0005 [b] 0.0010		
7	Old rum, 1902	100.5	0.054	0.061			34	33
8	Old rum, 1899	114.8	0.078	0.146			34	55
9	Old rum, 1896	115.3	0.078	0.171	65	[b] 0.0045	34	55
10	Old rum, 1893	114.7	0.082	0.162	65	[b] 0.0045	42	58
11	Rums bought from retail dealers.	100.0	0.042	0.088	55	[b] 0.0022	11	None.
12		68.3	0.018	0.022	15	[b] 0.0003	11	None.
13		89.7	0.007	0.032	30	[b] 0.0003	11	None.
14		93.9	0.023	0.156			11	None.
15		86.7	0.011	0.150			11	None.
	[Proof spirit + caramel to old rum tint.]						11	None.

[a] Furfurol determinations made on original liquor.
[b] Furfurol determinations made on distillate.
[c] Much CO_2 present.

REPORT OF B. M. PILHASHY, CINCINNATI, OHIO.

The figures given in the following table are for 100 cc, 100 proof. The extracts in both samples agree closely as do the acidity and ethers. The ethers were determined first in the original goods, the acidity being first neutralized. The titration was made with phenolphthalein, and close readings were difficult. Determinations were then made by distilling as for volatile acids and ethers in wine. The difference is quite apparent and, in the writer's opinion, can hardly be due to the indicator.

Results by B. M. Pilhashy.

[Figures for 100 cc, 100 proof.]

Date of inspection and proof.	Extract.	In N/10 KOH.		Copper reduced.		In N/10 KOH.	
		Acidity.	Ethers.	In sample.	In extract.	Acidity after distillation.	Ethers after distillation.
	Grams.	*cc.*	*cc.*	*Grams.*	*Grams.*	*cc.*	*cc.*
February, 1900 (proof 108.6)	0.1401	11.0	17.6	0.0491	0.0855	4.7	8.1
October, 1900 (proof 109.0)	.1400	10.7	19.7	.0618	.0806	3.6	8.0

The difference between the copper reduced in the original liquor and in the extract of the same is noticeable. The reverse was expected, the difference to be attributed to aldehyde reduction.

These results are hardly to be believed, and the work will be repeated as soon as possible. Twenty-five cubic centimeters, or the extract from an equivalent amount, were boiled for 35 minutes with Fehling's solution. This method was tried to see if a reduction figure could be applied to the solid and the liquid parts separately Fusel oil was not determined, owing to the breaking of the apparatus.

The following are figures for new goods, i. e., high wines as barreled at 101 proof:

Results obtained on new goods, 101 proof.

Date.	Acidity (cc N/10 KOH).	Ethers (cc N/10 KOH).	Fusel oil.
			cc per 100 cc.
May 25	1.8	13.6	1.266
May 26	2.1	15.4	1.30
May 27	2.0	13.0	.....
May 28	2.2	13.6	.....
May 29	2.6	11.2	1.366
May 30	1.8	10.8	1.63
June 1	2.2	12.4	[a]1.26
June 2	2.6	11.3	1.13
June 3	2.2	12.4	1.20
June 4	2.2	12.2	1.23
June 5	2.3	12.4	1.76
June 6	2.0	10.6	1.25
June 7	2.2	14.2	1.28
June 9	2.4	13.0	1.38
June 10	2.4	11.6	1.00
June 11	2.8	6.3	1.11
June 12	2.4	7.6	1.10
June 13	2.0	7.2	1.36

[a] No copper reduction; in 2 other samples no copper reduction was obtained.

In the fusel oil determinations 21.42 cc was deducted as the figures for the CHC_3+ C_2H_6O increase. This is taken from a bulletin and not verified.

It was the intention of the referee to report upon a line of experimental work which has been carried on in his laboratory for the past five years and which formed the subject of a preliminary communication to the American Chemical Society at its last annual meeting. Pressure of work in other lines, however, has prevented the completion and tabulation of results, which must be postponed for another year. This is regretted, as there are many points which will probably have a bearing upon methods of analysis when worked out, although the line of experiment is primarily in the direction of the establishment of composition standards, and is not a comparison or critical study of methods.

A careful study of the provisional methods prescribed by the association for the analysis of distilled liquors has convinced the writer of the desirability of modifying them in two or three of the principal determinations, and if this can be satisfactorily accomplished it will probably be sufficient progress for the present meeting.

The determination of alcohol, for instance, seems to be radically defective in not providing for a higher dilution of the liquor before distillation. As it stands, the method would give a distillate containing about 25 per cent of alcohol, which is entirely too strong and must inevitably lead to loss of alcohol. The amount taken for the determination should be cut down at least one-half, to 25 cc.

The referee has been accustomed to weigh rather than to measure the portion taken, using glass-stoppered weighing flasks. This is done in all cases where careful work is required, and only in this way can close agreement in results be attained.

It is therefore recommended that the method as given in the provisional methods for the analysis of foods[a] be modified to read as follows:

(2) Determination of alcohol: Weigh, or measure (at 15.6° C.) into a distilling flask 20 to 25 cc of the sample, dilute with 100 cc of water, and proceed as directed on page 82. The sample of distilled liquor taken for the determination of alcohol is diluted more than in the case of wine, because of the errors attending distillates high in alcohol, errors due to evaporation and to making up to volume at temperatures varying slightly from 15.6° C. All measurements must be made at about that temperature.

The next determination, that of extract, seems to be a little misleading, as the solid residue left on evaporation by most spirits is too small in quantity to become sirupy in consistence. The following is therefore recommended as a substitute under "3. Determination of extract," page 96.

Weigh, or measure (at 15.6° C.) 100 cc, evaporate nearly to dryness on the water bath, then transfer to a water oven and dry at the temperature of boiling water.

The determination of ash may be allowed to stand.

In the determination of acidity, the recommendation of Mr. Sawyer for a change from sodium hydroxid to a barium hydrate solution, for titration, is deserving of consideration. While it has not been tested by the referee, the change is recommended on account of the advantages Mr. Sawyer found it to possess. Therefore, substitute the following, under "3. Determination of acidity," on page 96:

Titrate 100 cc (or 50 cc diluted to 100 cc if the sample is dark in color) with decinormal barium hydrate, using phenolphthalein as indicator. The number of cubic centimeters employed is multiplied by 0.006 for the acidity expressed in grams of acetic acid per 100 cc.

In the determination of fusel oil, a change should be made which has been adopted in all official methods abroad, viz, the distillation of the sample with alkali to remove free acid and saponify esters.

I would recommend the substitution of the following for the third paragraph of the description, page 96:

Add a small quantity of alkali to 200 cc of the sample under examination, and distill slowly, till about 175 cc have passed over; allow the distilling flask to cool, add 25 cc of water, and distill again till the total distillate measures 200 cc. Dilute the distillate to exactly 30 per cent by volume (sp. gr. 0.96541 at 15.6°).

The method for the determination of ethereal salts, should be changed. The paragraph is vague, and in parts incorrect. Titration of the excess of mineral acid is not mentioned and no allowance is made for the subtraction of the volatile from the total acids. As the method stands, the calculation would give not only the excess of mineral acid added, but also both the free and combined acids in the sample.

Further, it might be questioned whether all of the ethereal salts distill at a temperature sufficiently low as to be found in the alcoholic distillate. I would recommend the substitution of the following, on page 98:

(9) Determination of ethereal salts: Neutralize the residue left after distillation in the fusel oil determination with N/10 H_2SO_4 and add an excess of 10 cc of the acid. Let stand five minutes, and make up to 200 cc. Titrate 2 portions of 25 cc each, using as indicators methyl orange in the first and phenolphthalein in the second. The difference gives the amount of alkali necessary to neutralize the organic acids in 25 cc of the sample. By subtracting from this figure the number of cubic centimeters of alkali required for the free acids and multiplying the result by 0.0088, the number of grams of ethereal salts (calculated as ethyl acetate) in 25 cc of the sample is determined.

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[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. No. 65, p. 96.

For next year's work I would suggest that the reporter make a comparative study of other methods of fusel oil determination, such as the Allen-Marquardt and Beckmann. The official (Roese) method is a very tedious, delicate operation, and the results are far from satisfactory. It is very desirable that some better method should be obtained.

About all the valuable information concerning distilled liquors which has appeared in the journals during the past few years will be found in the following references:

Beckmann: Ueber die Bestimmung des fuselgehaltes alkoholischer Flussigkeiten. Zeit. Nahr. Genuss., 1899, **2**: 709.

Beckmann: Neuerungen zur Bestimmung des fuselgehaltes alkoholischer Flussigkeiten. Zeit. Nahr. Genuss., 1901, **4**: 1059.

Schidrowitz: Composition of whisky. J. Soc. Chem. Ind., 1902, **21**: 814.

Zega: Examination of brandies from damsons and grape marc. Chem. Zeit., 1901, **25**: (75) 793.

Hewitt: The retarding influence of aldehydes on the maturation of potable spirits. J. Soc. Chem. Ind., 1902, **21**: 1896.

Mr. Sawyer. Mr. President, I would like to have two or three minutes for discussing the report. In my letter to the referee I said that there were certain determinations under way in my investigation which presumably would be completed before this meeting. It is of them that I wish to speak.

In the determination of fusel oil it was found impossible to carry out the Roese method, and I believe that it will not be practicable to use it in the average laboratory on account of its temperature requirements. The Beckmann method, to which the referee has referred, I have subjected to a preliminary test, and so far as can now be seen it is going to prove very satisfactory. I have not tested it to see how the results check, but understand that it has been tried in that respect by a number of German investigators whose figures have corresponded admirably. My test of the method was to determine whether it can be used in the ordinary laboratory at such a rate of speed as to make it suitable for routine work, and a single worker was able to make four determinations in an afternoon, which is better speed than could be obtained with the Roese method.

I suggested to Mr. Crampton that there was likely to be a marked difference between the behaviors of genuine and factitious liquors in regard to their reaction for tannin. This body is a characteristic ingredient of the extract of genuine distilled liquors which have been aged in oak casks. When the venders of factitious goods learn this they may begin to add tannin together with the burnt sugar which they now use for coloring, but for the present a test with ferric salts should show a distinct difference between aged and imitation goods.

There has been a tendency of recent years to hold furfurol responsible for those ultra-injurious effects of new liquor for which fusel oil used to be blamed. I believe that we shall have to abandon this doctrine and look for a new scapegoat. In the first place, new liquor contains less furfurol than old liquor does. In testing many samples of rum to see whether it disappears during the aging process, it was found that new goods contained a little, coming no doubt from the

breaking down of pentosans in the molasses, which is our raw material. In rum from uncharred packages—that is, in plain oak casks—the amount was about the same as in new goods. Now, rum of this sort is not esteemed for drinking. The best liquor—that which is properly matured for drinking—is put away for years in oak packages which have been charred inside. And it is in this best grade of liquor, whose noxious effects are much less than those of the newer goods, that the proportion of furfurol is highest. Its source is obvious. It comes from the dry distillation of the oak wood which accompanies the charring of the barrels.

My own experience with furfurol—and I worked for three years in an atmosphere laden with its vapors—leads me to believe that it is not nearly so toxic as is generally supposed. Furthermore, I believe that it is to the more volatile constituents that new and factitious liquors owe their specially objectionable qualities. The disagreeable experiences which I have had in working with them have convinced me that we should set about determining what they are and formulating methods for their estimation.

Mr. Bigelow. There is no report on vinegar, but I wish to suggest a modification of the method for the determination of caramel as given in Bulletin No. 65, page 65, under "Detection of coloring matters." The presence of the precipitate obtained is not sufficient proof of the presence of caramel; its character must also be considered. I will draw up an amendment to the present method embodying this idea and submit it to the committee on recommendations.

The President. The subject of spices will now be considered.

REPORT ON SPICES.

By R. E. Doolittle, *Associate Referee.*

No work of particular interest on the methods for the analysis of spices has been done during the past year, so far as my readings and observations have extended. The referee reported that no one volunteered for collaboratory work on the subject in response to the circular sent out by him. Before receiving notice of my appointment as associate referee, samples of the various varieties of whole pepper had been obtained from the leading importing houses for the purpose of investigating their composition, more particularly with reference to the enforcement of certain statutes embraced in the food laws of the State of Michigan. During the year these analyses have been completed, following in general the methods adopted provisionally by the association, and the few comments made in this report are based upon my own observations during this work. There is one matter of importance in discussing the methods for the analysis of spices, and that is, many States have standards fixed by statute for one or more of the spices, these standards being based on the methods now adopted provisionally by this association. Further, as these methods were very carefully investigated and compiled by Mr. Winton, whom we all know to be our best authority on the subject, we should go very slowly in making any radical change.

In the determination of starch by the diastase method, an animal diastase manufactured by Frederick Stearns & Co. has been adopted in our laboratory in place of

the powdered malt. This is a pancreatic diastase which gives no reduction on the Fehling solution, thereby eliminating that source of error and increase of work, and it is used exclusively in starch determinations at the University of Michigan. It is suggested that its use be investigated by the associate referee on spices for the coming year.

For determining the amount of copper reduced, I prefer weighing as cuprous oxid, as described by Munson under Methods for Determining Reducing Sugars.[a] The asbestos is prepared by cutting the best quality of woolly asbestos into fine pieces, digesting first with 1:3 hydrochloric acid and then with Fehling solution. The asbestos is packed into the Gooches by aid of a blunt glass rod, making a mat about one-fourth of an inch thick and thoroughly washed with hot water, then with 10 cc of alcohol, and then with 10 cc of ether. The Gooches thus prepared are dried in the oven at 100° C. for thirty minutes, allowed to cool one-half hour, and weighed. The cuprous oxid is collected, washed in the same manner with hot water, alcohol, and ether, and dried for thirty minutes at 100° C. and weighed after cooling for thirty minutes. The cuprous oxid may be dissolved off with hot dilute nitric acid and the same Gooch used for several determinations.

It is also suggested that in the determination of crude fiber the various kinds of filters and containers suggested in the provisional method[b] be thoroughly studied by collaborative work and the most serviceable plan reported. In our laboratory we conduct the boiling in 600 cc beakers covered with a watch glass, filter through linen after the acid treatment, and on weighed S & S blue ribbon filters after the alkali, washing thoroughly with hot water, then with a little alcohol, and then with ether, introducing the necessary correction for loss on treatment of the filter with the alkali, alcohol, and ether.

Mr. Doolittle. As Mr. Winton is not here, I suggest that the discussion be postponed until he is present.

Mr. Bigelow. There is no referee's report on meat, and as the only work done outside of our own laboratory is that reported by Mr. Grindley and is devoted chiefly to proteids, I suggest that his paper be read when the subject of nitrogen is discussed.

The President. Acting on this suggestion, the next report to be received will be that of the associate referee on dairy products considered from the standpoint of adulteration.

REPORT ON DAIRY PRODUCTS.

By Albert E. Leach, *Associate referee.*

In my report I have chiefly to recommend a few methods to supplement those already included in the provisional methods as published in Bulletin 65 of the Bureau of Chemistry, with a brief statement of reasons, in some cases, for their insertion.

Milk Preservatives.

The hydrochloric acid and ferric chlorid method for detecting formaldehyde, not only in milk, but also in other food products using pure milk as a reagent, has been so thoroughly tested by over six years of constant trial that it would seem to be worthy of a place among our provisional methods. In point of delicacy it is far

[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. No. 73. Proceedings of the nineteenth annual convention of the Association of Official Agricultural Chemists, p. 59.

[b] U. S. Dept. Agr., Bureau of Chemistry, Bul. No. 65, p. 154.

superior to the sulphuric acid test. I believe nothing but formaldehyde has yet been found that will give this peculiar and unmistakable color reaction.

While salicylic and benzoic acids are not ideal milk preservatives, their presence is always to be looked for, and the following methods[a] for their detection in milk are submitted.

Benzoic acid.—Add 5 cc of dilute hydrochloric acid to 50 cc of the milk in a flask and shake to curdle. Then add 150 cc of ether, cork the flask and shake well. Break up the emulsion which forms, by aid of a centrifuge, or, if the latter is not available, extract the curdled milk by gently shaking with successive portions of ether, avoiding the formation of an emulsion.[b] Transfer the ether extract (evaporated to small volume, if large in bulk) to a separatory funnel and separate the benzoic acid from the fat by shaking out with dilute ammonia, which takes out the former as ammonium benzoate. Evaporate the ammonia solution in a dish over the water bath till all free ammonia has disappeared, but before getting to dryness add a few drops of ferric chlorid reagent. The characteristic flesh-colored precipitate indicates benzoic acid. Care should be taken not to add the ferric chlorid till all the ammonia has been driven off, otherwise a precipitate of ferric hydrate is formed.

Salicylic acid.—Proceed exactly as directed for benzoic acid. On applying the ferric chlorid to the solution after evaporation of the ammonia, the well-known violet color indicates salicylic acid when present.

These methods for salicylic and benzoic acids while especially applicable to milk, from which the ether extracts both fat and preservative, are useful also with modifications for many other food products. The extraction of the ether solution with dilute ammonia, whereby the preservative is removed, permits the subsequent recovery of the ether by distillation.

Detection of Color in Butter.

Annatto is very readily detected when present in notable amounts in butter, and when the straw color due thereto is concentrated permanently on a piece of filter paper, as in the test given below, there is no reason for mistaking it. With the azo and other coal-tar dyes the case is not so simple, and it would seem as if a number of the best methods for testing these colors in butter should be placed among the provisional methods, so that the analyst may base his opinion on the result of a variety of tests whether positive or negative. It is hardly safe to condemn a sample on a single test or method.

Method of the Massachusetts board of health for annatto.—Treat 2 or 3 grams of the melted and filtered fat (freed from salt and water) with warm, dilute sodium hydroxid, and, after stirring, pour the mixture while warm upon a wet filter; a hot funnel may be used to advantage. If annatto is present, the filter will absorb the color, so that when the fat is washed off by a gentle stream of water the paper will be dyed straw color. It is well to pass the warm alkaline filtrate two or three times through the fat on the filter to insure removal of the color. If, after drying the filter, the color turns pink on application of a drop of stannous chlorid solution, the presence of annatto is assured.

Color in Oleomargarin.

The question of natural color in yellow oleomargarin does not vitally concern the analysts in Massachusetts by reason of certain recent court decisions, according to

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[a] Leach. An. Rep. Mass. State Board of Health, 1902, p. 475. Food and Drug Reprint, p. 23.

[b] A volume of ether largely in excess over that of the curdled milk has been found to be less apt to form an obstinate emulsion.

which oleomargarin, even though resembling yellow butter, can be sold with impunity if it does not contain a foreign dyestuff—that is to say, if the color is due entirely to the natural oils which it contains. For this reason very little attention has been given to the presence of palm oil, which in some sections of the country seems to have caused considerable trouble.

Oleomargarin without any color other than that inherent in the fat ingredients is now commonly found on the market, and, though devoid of dyestuffs, it rivals in depth of color butter which has been dyed. Frequent samples of this kind have been examined in our laboratory. On account of the processes to which cotton-seed oil and oleo oil are now submitted, with a view to enhancing their deep color, it is quite possible to obtain a very yellow oleomargarin without even resorting to the use of palm oil.

Most of the methods suggested for the detection of small quantities of palm oil in oleomargarin have not proved to be reliable. A number of such methods have been submitted by Mr. Ansil Moffatt, of Indianapolis, and while the writer has been unable, through lack of time, to personally test these methods, Mr. A. H. Gill, of the Massachusetts Institute of Technology, has tried them, and one of them, namely, the aluminum chlorid test, proved quite satisfactory in his hands. This test was carried out according to Mr. Moffatt's directions, as follows:

A solution of 10 per cent anhydrous aluminum chlorid in methyl alcohol is taken as a reagent. The clear filtered fat is put into a test tube, a few drops of the above reagent are added, and the test tube is heated with shaking until the solvent is evaporated and the alumina precipitated. The presence of palm oil is indicated by the color of the precipitate, which varies with the character of the oil contained in the mixture. Comparative tests had best be made on samples of known purity, both of pure oil and of mixtures. The following letter was received from Mr. Gill in regard to these tests:

Regarding my experience in the use of the so-called "cookery test" of Mr. Moffatt, I am willing to make the following statements: Cotton-seed oil and oleomargarin containing 1 per cent or less of palm oil show a decided coloration when tested with aluminum chlorid in alcohol. Had I another test as decided as this, or had I made a careful series of tests with other deeply colored oils, I should be willing to accept it as conclusive proof of the presence of palm oil. From my experience in oil testing, I am unwilling to condemn an oil upon the results of one test, particularly if it be a color test. I have tried both the tests employing Nessler's solution and an alcohol and sulphuric acid test upon similar mixtures of palm with other oils without obtaining results which were satisfactory. I can not, therefore, indorse these tests.

Preservatives in Butter.

In view of the fact that boric acid is commonly found in butter, a method for its detection therein should be provided. The following is submitted:

Detection of boric acid in butter.—Melt about 25 grams of the sample on the water bath, pour off the fat from the aqueous solution that settles to the bottom of the container, acidify the aqueous solution slightly with hydrochloric acid, and test in the usual manner with turmeric paper for boric acid.

Fat in Cheese.

During the past year a large number of cheese samples have been analyzed in the department of food and drug inspection of the Massachusetts board of health, and the following modification of the Babcock process for determining fat has been used to advantage:

Determination of fat in cheese—Lythgoe's modification of the Babcock method.—Weigh accurately about 6 grams of the sample in a tared beaker. Add 10 cc of boiling water and stir with a rod till the cheese softens and an even emulsion is formed, preferably adding a few drops of strong ammonia to aid in the softening and emulsion-

izing, and keeping the beaker in hot water till the emulsion is tolerably complete and free from lumps.

If the sample is a full-cream cheese, a Babcock cream bottle is employed. The contents of the beaker, after cooling, are transferred to the test bottle as follows: Add to the contents of the beaker about half of the 17.6 cc of sulphuric acid regularly used for the test, stir with a rod, and pour carefully into the bottle, using the remainder of the acid in two portions for washing out the beaker. Finally, proceed as in the Babcock test for milk. Multiply the fat reading by 18 and divide by the weight of the sample taken, to obtain the per cent of fat.

Condensed Milk.

It would seem that methods for the analysis of sugar-preserved condensed milk should properly be included under dairy products, and the following methods, which have been in constant use in Massachusetts for over eight years, are submitted:

Preparation of the sample.—Mix thoroughly by transferring the contents of the can to a large evaporating dish and working it with a pestle until homogeneous. Weigh 40 grams of the mixed sample in a 100 cc sugar flask, or transfer thereto by washing, and make up to the mark with water.

Total solids—Dilute a measured portion of the above 40 per cent solution with an equal amount of water, and transfer by a pipette 5 cc of the diluted mixture, corresponding to 1 gram of the condensed milk, into a tared platinum dish, which is allowed to remain in contact with the live steam of a water bath for at least two hours after the last traces of water have been evaporated to leave an apparently dry residue. Transfer to a desiccator, cool and weigh.

Ash.—Carefully ignite the residue from the total solids, cool and weigh.

Fat.[a]—Measure 15 cc of the above 40 per cent solution, corresponding to 6 grams of the condensed milk, into a Babcock test bottle. Fill nearly to the neck with water, add 4 cc of Fehling's copper solution, and shake thoroughly and rapidly, separating the precipitated proteids and fat by means of a centrifuge,[b] or the precipitate may be allowed to settle of itself, which it does more quickly in the cold. Withdraw the supernatant, sugar-containing liquid by means of a small-stemed pipette with a wisp of wet absorbent cotton twisted over the bottom to serve as a filter. Wipe off the cotton into the bottle on withdrawing the pipette. Give the precipitated proteids and fat two additional washings, as above, by shaking with water, separating the precipitate, and removing the washings with the pipette. If the precipitate is caked hard after centrifuging, use a stiff platinum wire as a stirrer. Finally, add water to an approximate volume of 17.5 cc and 17.5 cc of sulphuric acid, and continue the test as in the Babcock process of milk testing, multiplying the reading by 3 for the percentage of fat in the sample.

Proteids.—Dilute 5 cc of the 40 per cent solution, corresponding to 2 grams of the sample, to about 40 cc and add 0.6 cc of Fehling's copper solution. Nearly neutralize with sodium hydroxid stopping just short of alkalinity. Pass through a weighed filter paper, wash, dry at 100° C., and weigh. Burn the precipitate in a porcelain crucible, the difference between the weight of the dry precipitate and the weight of the ash being the weight of the proteids and fat. Expressing this in percentage and deducting the per cent of fat previously obtained, the result is the per cent of proteids.

Milk sugar.—Make up the filtrate and washings from the previous operation to 100 cc and determine the lactose either gravimetrically or volumetrically in this solu-

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[a] Leach. J. Am. Chem. Soc. 1900, **22**: 589.

[b] While the steam-driven centrifuge may be used for this, it is better to centrifuge in the cold, since the heat of the steam-driven machine cakes the precipitate so that it is harder to wash.

tion. If the volumetric method is used and the solution is of the exact strength directed above, milk sugar may be calculated as follows:

$$\frac{100\times 0.067}{S\times 0.02}=L,$$

where L is the per cent of lactose and S the number of cubic centimeters of milk solution prepared as above necessary to reduce 10 cc of Fehling's solution.

Cane sugar.—Determine by difference, deducting the milk solids (milk sugar + proteids + fat + ash) from the total solids.

Adulteration.—The chief form of adulteration in condensed milk consists in the employment of skim milk in its manufacture. The nature and extent of the adulteration is not always apparent from the mere content of fat actually found in the sample, since this is to some extent dependent on the amount of condensation. Thus the most useful factor in judging of the purity of a sample is really the fat in the original milk. To calculate this it has hitherto been necessary to make a complete analysis of the sample to obtain the percentage of milk solids not fat. Divide this figure by an assumed standard for solids not fat (preferably basing this figure on the standard fixed by law), and the result expresses the "number of times condensed." Finally dividing the percentage of fat in the condensed milk by the "number of times condensed" the "fat in the original milk" is obtained.

We have recently adopted a simpler method for the calculation of fat in the original milk which involves merely the determination of fat and ash in the sample.[a] Assuming 0.70 per cent as the ash of pure milk, the factor representing the "number of times condensed" is found by dividing the ash of the condensed milk by 0.7. The "fat in the original milk," as thus calculated, is of course an arbitrary factor, and is useful only in deciding whether or not skimmed milk has been used in preparing the sample. By assuming the highest reasonable figure for the ash of natural milk, it is readily seen that the highest result is obtained for the fat in the original milk, and hence the benefit of any doubt is given to the manufacturer.

The ash of the condensed milk sample is conveniently obtained by evaporating to dryness 12.5 cc of the 40 per cent aqueous solution of the sample which forms the basis of the various determinations, this volume corresponding to 5 grams of the sample. The residue is then incinerated in the muffle, cooled and weighed. If the fat in the original milk as above calculated is found to be much below 3 per cent, there is evidence to show that skimmed milk has been used. This method of procedure is considered more accurate than that hitherto adopted for calculating the fat in the original milk, since the milk ash is a more constant factor than the percentage of solids not fat, on which the old method was based.

Recommendations.

I would recommend for provisional adoption the following methods: Under "Milk:"

The hydrochloric acid and ferric chlorid method for formaldehyde in milk.[b]—To 10 cc of milk in a porcelain casserole add an equal volume of concentrated hydrochloric acid containing 1 cc of 10 per cent ferric chlorid solution to each 500 cc of acid. Heat nearly to the boiling point over the free flame, holding the casserole by the handle and giving it a rotary motion to break up the curd. A violet coloration indicates formaldehyde.

Also the methods for salicylic and benzoic acids given in the opening of this report.

Under "Butter" the method of the Massachusetts board of health for annatto

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[a] An. Rep. Mass. State Board of Health, 1902, p. 475. Food and Drug Reprint, p. 23.
[b] An. Rep. Mass. State Board of Health, 1897, p. 558. Food and Drug Reprint, p. 20.

given in this report. And also the methods of Geisler, Low, and Doolittle for detection of colors, as follows:

Geisler's method for azo colors.[a]—A few drops of the clarified fat are spread out on a porcelain surface and a pinch of fuller's earth added. In the presence of various azo dyes a pink to violet-red coloration will be produced in a few minutes. Some varieties of fuller's earth react much more readily than others with azo colors.

Low's method for azo colors.[b]—A small amount of the material to be tested is melted in a test tube, an equal volume of a mixture of one part of concentrated sulphuric acid and four parts of glacial acetic acid is added, and the tube is heated nearly to the boiling point, the contents being thoroughly mixed by shaking; the tube is then set aside, and after the acid solution has settled out it will be found to be colored wine-red in the presence of azo color, while with pure butter-fat comparatively no color will be produced.

Doolittle's method for azo dyes and annatto.[c]—The melted sample is first filtered. Two test tubes are taken and into each are poured about 2 grams of the filtered fat which is dissolved in ether. Into one test tube 1 or 2 cc of dilute hydrochloric acid are poured, and into the other about the same volume of dilute potassium hydroxid solution. Both tubes are well shaken and allowed to stand. In the presence of azo dye, the test tube to which the acid has been added will show a pink to wine-red coloration, while the potash solution in the other tube will show no color. If annatto has been used, on the other hand, the potash solution will be colored yellow, while no color will be apparent in the acid solution.

I would further recommend for provisional adoption under "Butter" the method for boric acid in the early part of this report, and under "Cheese," the modified Babcock method given for detecting fat.

And, finally, I recommend that the scheme for analysis of condensed milk as given in this report be provisionally adopted.

The PRESIDENT. Is there any discussion on the paper?

Mr. PATRICK. I am not sure that I understood the method proposed by Mr. Leach for determining proteids in condensed milk. Was it to precipitate with copper hydrate, dry, ignite, and take the loss as proteids——

Mr. LEACH. Weighing before ignition.

Mr. PATRICK. Is Mr. Leach sure that this method gives correct results? I am under the impression that it has been found lacking in accuracy, for the reason that the drying of the copper precipitate does not expel all the water.

Mr. LEACH. I think we have pretty thoroughly tested its accuracy, having used it in the analysis of many hundreds of samples during the past eight years. If you are careful in drying and keep the temperature limit between 100° and 105° good results are obtained, at least the error will be a very small one. The quickness of making the test also had something to do with our adoption of that method.

The PRESIDENT. The next subject for consideration is cocoa products.

Mr. BIGELOW. Mr. Eaton has submitted the first report on this subject, but, as has been the custom in regard to the other methods, it will be printed and distributed to the associates on food adulteration for informal criticism before it is submitted to the association for adoption as a provisional method.

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[a] J. Am. Chem. Soc. 1898, **20**: 110.
[b] J. Am. Chem. Soc., **20**: 889.
[c] U. S. Dept. of Agr., Bureau of Chemistry Bul. 65, p. 152.

The PRESIDENT. The report on preservatives is in order.

REPORT ON PRESERVATIVES.

By W. D. BIGELOW, *Referee.*

Although the referee had the promise of considerable collaboration in the study of preservatives, and some work was begun, no definite results have been obtained. There is probably no field in connection with food chemistry which is in greater need of further study than that of the determination of preservatives.

The detection of some chemical preservatives is a very difficult matter because of the lack of reactions sufficiently delicate and characteristic to indicate their presence in the quantity in which they are used. It is certainly true that preservatives sometimes exist in foods which are reported by food laboratories as being free from them. On the other hand, there is a grave danger of error in the other direction, and it is believed by many that preservatives have been reported in samples in which they really did not occur, owing to the misinterpretation of certain class reactions and the giving of too much weight to reactions which were only sufficient to indicate "traces."

There is a tendency in some States to require by law that where preservatives are employed the kind and amount present should be stated on the label. In view of this fact it will probably be important in the near future to determine as nearly as possible the amount of preservatives present in foods. The methods that have been suggested heretofore for this purpose are inadequate.

During the last year considerable attention has been given to qualitative methods for the detection of formaldehyde and quantitative methods for the determination of salicylic and benzoic acid. It is intended to continue the work during the coming year and to extend it as far as the time and amount of collaboration available will permit.

The referee desires to emphasize the importance of this work and to request the collaboration of as many members of the association as possible.

The PRESIDENT. Mr. Winton is present and we shall be glad to hear the report on flavoring extracts.

Mr. WINTON. I wish first to report on the committee's visit to the Secretary of Agriculture. I will take this opportunity to say that the Secretary received us very cordially, and agreed to address the meeting later in the day or perhaps early to-morrow. Knowing this, I hope we shall have a large attendance.

I have no written report to make on flavoring extracts, but will submit the following remarks:

REPORT ON FLAVORING EXTRACTS.

By A. L. WINTON, *Associate Referee.*

Mr. Mitchell, the associate referee last year, outlined very good methods indeed, which have needed only slight changes. It is not necessary, I think, to go into those changes at the present time. There are, however, certain new lines of work which must be undertaken sometime in the near future. One subject in particular has been brought very forcibly to our attention during the past year by work done in the State of Michigan. It is not necessary for me to go into this subject in detail, as Mr. Doolittle is here and will give us full particulars of the case.

The matter referred to concerned lemon extract. It has been the custom of some manufacturers to prepare a cheap grade of lemon extract containing only a trace of

lemon oil and but a fraction of the amount of alcohol prescribed by the United States Pharmacopœia. As the cost of alcohol in a good extract is several times as great as the cost of the other ingredients, the purpose of the manufacturer apparently is to reduce the amount of alcohol as much as possible. He does this by shaking the lemon oil with weak alcohol. The alcohol is too weak to dissolve any appreciable amount of the terpenes, but it does dissolve a certain amount of the citral and possibly other oxygenated constituents, and as a consequence the weak alcoholic liquid, after being separated from the undissolved oil, has a lemon odor. It has been the claim of these manufacturers and certain chemists that this "washed out" extract is equal or superior to that made by the old process, because the terpenes, which they regard as valueless, are eliminated.

The supreme court of Michigan has rather forced us to consider this matter, because while they have accepted the United States Pharmacopœia as the standard they have ruled that manufacturers have the privilege of improving on the processes of the Pharmacopœia. It is my opinion—and in this matter I have worked in thorough accord with Mr. Doolittle, of Michigan—that the citral is not by any means the only valuable ingredient of lemon extract, but that the terpenes also have decided value. I think those who have tried both the washed out and the genuine U. S. P. extracts in food products will agree that the flavor is somewhat different. While the terpenes of themselves may not be so aromatic as the citral, still in combination with the citral they give a blended flavor which is the true flavor of lemon. Either one by itself is not sufficient. It has been my custom to compare the part played in the extract by limonene, the chief terpene of lemon oil, to the low pipes of the organ. These low tones of themselves are not particularly agreeable, but combined with the high tones they produce the indescribable depth and grandeur of organ music. So in the case of lemon extract, the limonene alone is not highly aromatic, but blended with the citral it contributes strength or body to the flavor. While it is my firm belief, based upon considerable investigation, that the value of an extract is not determined by the citral alone, at the same time I think it is highly desirable that a good method for determining citral be decided upon, if for no other purpose than to assay extracts made with a washed out oil, which is even a greater fraud than making extracts by the washing out process. Mr. Doolittle investigated this matter thoroughly, and I hope he will talk to us on the subject.

There is another line of work which must be taken up eventually, and that is the examination of the mixtures of so-called fruit ethers made in imitation of various fruit flavors, such as strawberry, raspberry, and banana.

The cheap soda-water sirups are very largely made out of these mixtures of ethers with coal-tar dyes and other ingredients, many of them containing no genuine fruit extract or fruit juice whatever. Anyone who has had any experience with ice cream or soda water purchased at watering places knows that by the palate alone one can very quickly distinguish between the artificial and the genuine. What we need now are better chemical methods for their detection. Of course these artificial extracts of strawberry or raspberry must be colored in order to pass for the true fruit sirups. Almost always the artificial color can be detected and that at once brands the article as not being made entirely from the fruit; but the difficulty is that the genuine products are also colored so the presence of an artificial coloring matter does not tell us whether the product is purely an artificial mixture or whether it is a genuine fruit product which has been colored to improve its appearance.

Our methods for vanilla extracts, with slight changes, are quite satisfactory. We have certain difficulties to contend with, to be sure, but many of them are being overcome by analytical research. For example, there is no way of determining whether the vanillin in vanilla extract is from the genuine bean or from the synthetic product. The determination of vanillin is usually of almost no value in determining adulteration except in rare cases where none at all or an abnormally large

amount is present. If prepared according to the United States Pharmacopœia vanilla extract contains about 0.2 per cent of vanillin while some of the cheapest extracts examined contained 0.6 per cent. In most cases the examination for coloring matter aids greatly in discriminating between genuine and artificial extracts. On the whole, our methods for examining vanilla extract are fairly satisfactory, as, indeed, are the methods for lemon extract with the exception of the one point—the determination of citral.

Mr. Doolittle. Mr. Winton has quite thoroughly covered the chemistry of the lemon extract controversy. The facts of the particular case referred to were as follows: When this subject came up in the courts of Michigan we were confronted by expert testimony from the State universities of Michigan and Wisconsin, and it developed in the course of the trial that in those States a product called terpeneless extract of lemon was being made. That is, the manufacturers claimed to extract from lemon oil only the flavoring properties, leaving the hydrocarbon portion behind. They claim that by agitating a 5 per cent solution of oil with a dilute alcoholic solution for a given length of time, and repeating the agitating after standing, all the citral and other flavoring constituents of the oil are extracted. This case was carried to the supreme court of the State of Michigan, and in the opinion handed down the court held as follows: That the legislature had in mind, when the food law was formulated, the formula of the United States Pharmacopœia for the manufacture of lemon extract, and that this formula is the standard for lemon extract, but that this does not prevent any manufacturer from producing an extract by any improved process, provided that the extract contains all the valuable constituents, i. e., all the flavoring constituents of a 5 per cent solution of lemon oil. In this particular case the manufacturers admitted that they used only a 5 per cent solution of lemon oil, and as experiments show that it is impossible by any chemical process to remove all the citral and other flavoring constituents, producing a perfectly tasteless terpene, they admitted that the extract in question was not up to the standard as defined by the court, and so pleaded guilty. But they now claim to use a 10 per cent solution of lemon oil and by treating this by the method described to remove from the increased amount of oil enough citral and other flavoring ingredients to equal those present in a 5 per cent solution of the oil.

Another curious fact brought out during the trial illustrates what Mr. Winton has said, i. e., the manufacturers are making a terpeneless extract for Michigan, dissolving the residual oil in alcohol and selling it in Wisconsin for a 5 per cent solution, thus complying with the U. S. P. formula as is required in Wisconsin, and I do not believe that the analyst can detect the exhausted product. Our methods for the analysis of lemon extract depend upon the amount of hydrocarbons in the solution. What confronts us in Michigan, therefore, is the

need of a method for determining the amounts of citral in such alcohol solutions as lemon extract and also a method of qualitatively and quantitatively, if possible, determining the other oxygenated constituents of the oil that go to make up the flavor of the extract and are characteristic of lemon oil.

The PRESIDENT. Would it not bring relief if the standards were changed so as to conform to those of the adjoining States?

Mr. DOOLITTLE. That would not help us, as the manufacturers use this exhausted oil and still come up to the requirements of such standards.

Mr. LEACH. In Massachusetts we have been interested in investigating the people who have used the so-called terpeneless extracts without any oil whatever, and one manufacturer has taken the bull by the horns by trying to conform to our other statute which compels labeling all compound goods with the percentage of ingredients, and he words his label thus: "Avoid lemon extracts containing hydrocarbon compounds because they are apt to deteriorate. Take only extracts that have none of those objectionable ingredients. This is warranted to contain none."

Mr. DOOLITTLE. That is one of the strongest claims made and it is sustained by expert authority in Wisconsin and also by Doctor Vaughan, of Michigan. Not only that, but it is claimed that the use of strong alcohol is a very great detriment in the lemon extract because alcohol tends to curdle products containing milk or eggs.

Mr. FISCHER. I examined a lemon extract recently in Wisconsin which, when assayed by the precipitation method, gave 100 per cent lemon oil, but when assayed by the polarization method it showed only 15 per cent. It seems to have consisted of about 15 parts of so-called "robbed" oil, dissolved, and 85 parts of cotton-seed oil. Evidently the provisional methods are very much at fault in such a case. In assaying the oil of lemon it appears to me, without claiming to be an expert, to be extremely difficult to determine the amount of citral in the oil itself, and next to impossible to determine the amount of other oxygenated products; and when it comes to a 5 per cent solution the methods fail completely.

Mr. BROOKS. Does not lemon oil contain naturally these oxygenated products, and if the Pharmacopœia directs that the extract of lemon shall be a solution of oil of lemon, is there any way of evading that requirement? If the law is so worded as to require a solution of the whole oil, what claim can be made for a lemon extract made by using a robbed oil, or even the citral oil?

Mr. LEACH. I think there is a means of testing the separated oil, and am inclined to think there would be a difference in refraction between the so-called "robbed" oil, or, as we call it, the "float" oil, and the pure oil.

At 12.40 the convention adjourned until 1.30 o'clock p. m.

THURSDAY—AFTERNOON SESSION.

After some instructions to committees B and C on recommendations of referees, the president called for the report of the referee on foods and feeding stuffs, which was submitted by Mr. Van Slyke, acting for Mr. Fuller.

REPORT ON FOODS AND FEEDING STUFFS.

By F. D. Fuller, *Referee.*

In reply to a circular letter sent out inviting cooperation in the work for 1903, eleven chemists promised to assist. To these men the following instructions were sent:

Instructions for Work on Feeding Stuffs for the A. O. A. C., 1903.

Two samples are sent out this year for work on feeding stuffs.

No. I. Distiller's grains.

No. II. Wheat bran.

The following determinations are to be made upon both samples: Moisture, fat, and crude fiber.

Moisture is to be determined by the official method, Bulletin No. 46, Revised Edition, Department of Agriculture, page 23, or according to the method used in your laboratory. In reporting results state method employed.

Fat (ether extract) is to be determined by the official method, Bulletin No. 46, page 23, and also by the Dormeyer method. A description of the latter method is sent herewith.

Crude fiber is to be determined by the official method, Bulletin No. 46, page 26, by König's method, and also by using a modification of König's method. Inclosed please find a description of König's method, and also one of the proposed modification.

Please report results at your earliest convenience.

Yours truly,

F. D. Fuller, *Geneva, N. Y., Referee.*
L. H. Smith, *Urbana, Ill., Associate referee.*

It gives me pleasure to acknowledge the kindness of the J. W. Biles' Company, of Cincinnati, Ohio, who furnished a supply of Biles's Fourex distiller's grains for the use of the referee. The bran was taken from a lot in use at the New York experiment station, and previous to grinding 8.81 per cent of water was removed at 60° C.

Estimation of Moisture.

The moisture was not determined as a matter of investigation of methods, but simply to make it possible to calculate the other determinations to a water-free basis.

Table I.—*Moisture determination.*

Analyst.	Method of drying.	Distiller's grains.	Bran.
		Per cent.	*Per cent.*
E. B. Holland, Massachusetts	Glycerin and water oven, 100° C	7.38	7.09
H. J. Warner, U. S. Department of Agriculture.	Water oven, 5 hours	6.11	4.18
Do	Water oven, 22 hours	6.72	4.21
Do	In vacuo, 5 hours, 80° C	6.24	4.90
Do	In vacuo, 5 hours, 100° C	6.65	5.70
H. H. Hanson, Maine	Steam oven, 5 hours, 98° C	6.40	4.26
C. D. Howard, West Virginia	In vacuo, 2 hours	6.97	5.43
J. A. Bizzell, New York (Ithaca)	Official method	6.99	5.73
L. H. Smith and E. M. East, Illinois	Glycerin and water oven, in hydrogen, 5 hours, 104–105° C.	4.39	5.06
A. W. Bosworth, Rhode Island		7.04	4.94
C. W. Mudge, New York (Geneva)	Steam oven, 10 hours, 98° C	6.91	4.98

Estimation of Ether Extract.

According to the recommendation of the referee on foods and feeding stuffs for 1902, we have compared the Dormeyer method with the present official method of fat extraction.

In the Chemiker Zeitung[a] Beger gives some results obtained by digesting the materials in pepsin solution previous to extracting the fat. In all cases an additional quantity of fat was obtained after the pepsin treatment, the increase being greater when using materials rich in protein.

This process was first used by Dormeyer[b] and later studied by Nerking,[c] who argued that the ordinary extraction with ether could not remove all the fat, because it was chemically combined with the albuminoids "Fetteiweissverbindungen," and the compounds must be broken up and the fat liberated before it could be completely removed. Following is a description of the Dormeyer method:

Three to 5 grams of substance are digested with 1 gram of pepsin, Merck (this is found to be free from ether extract), dissolved in 480 cc water and 20 cc of 25 per cent hydrochloric acid at 37°–40° C. for twenty-four hours. The residue is then filtered in a Gooch crucible, washed several times with cold distilled water, and after drying is extracted with ether in the usual way. The filtrate must be shaken out with ether several times and the extract from this united with that from the dried residue.

Table II.—*Ether-extract determination.*

Method and analyst.	Distiller's grains.		Bran.	
	Original sample.	Water-free.	Original sample.	Water-free.
OFFICIAL METHOD.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
E. B. Holland, Massachusetts	10.67	11.52	5.05	5.53
	10.67	.....	5.18	.....
	.....	.....	5.19	.....
H. J. Warner, U. S. Department of Agriculture	10.13	10.84	5.12	5.43
	10.13	.....	5.20	.....
	10.14	.....	5.20	.....
H. H. Hanson, Maine	10.65	11.29	4.90	5.16
	10.49	.....	4.97	.....
J. A. Bizzell, New York (Ithaca)	10.84	11.61	5.25	5.53
	10.75	.....	5.17	.....
L. H. Smith and E. M. East, Illinois	10.44	10.85	5.22	5.47
	10.18	.....	5.19	.....
	10.30	.....	5.18	.....
	10.44	.....	5.17	.....
	10.51	.....	.....	.....
A. W. Bosworth, Rhode Island	15.92	[d]17.22	9.51	[d]10.05
	16.10	.....	9.59	.....
C. W. Mudge, New York (Geneva)	10.97	11.90	5.23	5.49
	11.13	.....	5.19	.....
	11.15	.....	5.25	.....
Average	.....	11.33	.....	5.43

[a] 1902, vol. 26, No. 11.

[b] Pflügers, Archiv. f. Physiologie, 1895, **61**: 341; ibid., 1896, **65**: 90.

[c] Pflügers, Archiv. f. Physiologie, 1901, **85**: 330.

[d] Result omitted from average.

TABLE II.—*Ether-extract determination*—Continued.

Method and analyst.	Distiller's grains.		Bran.	
	Original sample.	Water-free.	Original sample.	Water-free.
DORMEYER METHOD.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
E. B. Holland, Massachusetts	12.36	13.26	5.70	6.27
	12.19	.....	5.96	.....
H. J. Warner, U. S. Department of Agriculture	10.91	11.76	5.05	5.33
	11.03	.....	5.09	.....
	11.07	.....	5.11	.....
H. H. Hansen, Maine	11.77	11.87	4.85	5.07
	10.46	.....	.....	.....
J. A. Bizzell, New York (Ithaca)	11.49	12.32	5.14	5.55
	11.43	.....	5.31	.....
L. H. Smith and E. M. East, Illinois	8.42	10.01	4.50	5.37
	8.87	.....	4.99	.....
	10.20	.....	5.44	.....
	10.77	.....	5.48	.....
Average	.....	11.84	.....	5.52

NOTES AND COMMENTS OF ANALYSTS.

E. B. Holland.—After the digestion with pepsin the solution was filtered through linen and washed to a total volume of about 800 cc. This combined filtrate and washings were shaken out with ether three times in a separatory funnel, which cleared the solution considerably. The extract previous to the evaporation of the ether was of a white gelatinous appearance. The residue in the filter, after drying, was inclosed in a capsule and extracted as in the official method.

H. J. Warner.—Found the Dormeyer method long and tedious.

H. H. Hansen.—Much difficulty found in filtering after the digestion with pepsin. Much more time was consumed and greater care required with this method than with the official method. There is, moreover, much greater chance for error.

L. H. Smith and E. M. East.—A second extraction of 16 hours by the official method was made in every case. As only an insignificant amount of extract was obtained, we may conclude that 16 hours is ample time for the extraction. The results by the Dormeyer method are not satisfactory, and most of the results appear to be too low, as compared with those by the official method. The method, on the whole, does not appear to be practical.

COMMENTS OF REFEREE.

A glance at the foregoing table will show that the results obtained by the Dormeyer method are a little higher than those secured by the official method, greater differences being observed in the results from distillers' grains, a food which contains over twice as much crude protein as wheat bran, a fact which might readily lead one to suppose that some of the fat was combined with the albuminoids, as maintained by Nerking, and only needed a digestion with pepsin to bring it into a readily ether-soluble condition. However, the question which arises at this point is this: Is the additional extract obtained by the Dormeyer method a positive increase in the percentage of crude fat contained in the material under examination? We all know that the ether extract of the official method is already much too high, as it contains bodies which can not come under the head of crude fat, and that by extracting with petroleum ether we secure an extract which more nearly represents the fat content of foods. Therefore we do not care to increase the amount of ether extract unless we are abso-

lutely sure of making an addition of fatty bodies alone. Beger [a] made a few qualitative tests upon the extract obtained by the Dormeyer method and found present a fat which responded to the acrolein and refractometer tests, "fat spot," saponification, etc., but he made no examinations to prove the purity of the extract or to settle beyond doubt the exact nature of the fat present.

Had time permitted, it was the intention of the referee to make these particular points a subject of research. Your referee believes that valuable time is lost in preparing samples, sending them to various men for analysis, and attending to the necessary correspondence pertaining to the same, which otherwise might be used by the referee and his associate in conducting systematic research in the branch of work to which they may be assigned by the association. Therefore, I would recommend that next year the referee and associate devote a part of their time to research work in connection with the points mentioned above.

Estimation of Crude Fiber.

The official method for the estimation of crude fiber originated from the demand for a fiber which contains only a very small quantity of nitrogen. But since we have considered the pentosan content of foods it has been proven by König and many other analysts that the crude fiber obtained by the official method contains pentosans and in some materials they are present in considerable quantity. Since the official method does not give us a pentosan-free fiber it was the object of the referee to seek for a new method by which such a fiber may be prepared.

In 1900 the attention of the association was directed to a method by J. König [b] for the estimation of pentosan-free crude fiber. In 1901 Mr. Fraps presented a paper before the association in which he showed that the König method is superior to the official method, in that it yields a fiber which is practically free from pentosans. But he also showed that in the case of cotton-seed meal and other materials rich in nitrogenous bodies, the fiber obtained is much too high. Acting upon his suggestion, a study of the König method was taken up by the referee in 1902, who corroborated the results obtained by Mr. Fraps and showed, furthermore, that the König method, while removing the pentosans, does not yield a fiber low in nitrogen. Therefore he recommended that the present referee make a further study of this method with the additional treatment of the fiber with alkali, as in the official method. The König method was thus modified, and the results in Table IV are obtained by using the official, König, and König modified methods. Following is a description of the König method and also the modification, with such precautions and suggestions as seem necessary.

The König Method for Crude Fiber.

(*a*) *Glycerol-sulphuric acid.*—Determine the specific gravity of the glycerol by means of a picnometer or specific-gravity balance. A hydrometer can not be used,

[a] Chem. Ztg. 1902, No. 11.

[b] Analyst, **23**: 47.

as on glycerol its readings would not be correct. The per cent of glycerol may be calculated by the following table:

TABLE III.—*Calculation of glycerol from specific gravity.*

Specific gravity at 15.5° C.	Glycerol.	Specific gravity at 15.5° C.	Glycerol.
	Per cent.		*Per cent.*
1.2674	100	1.2460	92
1.2647	99	1.2433	91
1.2620	98	1.2406	90
1.2594	97	1.2380	89
1.2567	96	1.2353	88
1.2540	95	1.2327	87
1.2513	94	1.2300	86
1.2486	93		

Should the temperature of the specific gravity determination not be 15.5° C, a correction may be made by adding 0.00058 for each degree above the temperature.

Dilute the glycerol to 86 per cent, or 1.230 specific gravity, and make up a solution containing 20 cc sulphuric acid (1.84 specific gravity) per liter of the glycerol-acid mixture.

(*b*) *The determination.*—Place 3 grams of substance in a 500 cc Erlenmeyer flask, add 200 cc glycerol-sulphuric acid, and connect the flask with an inverted condenser, the tube of which passes only a short distance beyond the rubber stopper into the flask. Heat to boiling, and boil very gently for an hour, shaking the flask from time to time so as to wash down any particles. The boiling should take place in such a manner that only a few drops of water are condensed. Prepare a thin layer of asbestos in a 2-inch Hirsch funnel, and on this place a perforated platinum disk. Filter the glycerol on this, using a suction pump. The mixture should be shaken well before it is poured on the filter, and each flask should have the full benefit of the suction pump when the filtration starts until the glycerol begins to come through. Wash with hot water, with alcohol, and with a mixture of equal volumes of alcohol and ether. The alcohol and ether remove, besides fats, certain products not soluble in water formed by the action of the glycerol acid. Transfer to a platinum dish, dry, weigh, and incinerate completely. The loss in weight is crude fiber.

(*c*) *Precautions.*—A primary necessity is to have the glycerol of the required density. A comparatively slight variation in density will cause an increase or decrease in boiling point, with a corresponding decrease or increase in the crude fiber.

In filtering, considerable time is gained by using a flat-bottomed porcelain funnel, known in German laboratories as a "Nutsche." Dilute the hot glycerol-acid mixture with 300 cc of boiling water and filter through linen.

(*d*) *Modification of König's method.*—Treat as in the König method and after washing transfer the residue to an Erlenmeyer flask, using 200 cc of hot 1.25 per cent sodium hydrate solution. Attach a condenser and boil gently for 30 minutes. Filter and wash with hot water. Transfer the residue to a Gooch crucible, dry, weigh, and incinerate completely. The loss in weight is crude fiber.

TABLE IV.—*Crude fiber determination.*

Method and analyst.	Distiller's grains.		Bran.	
	Original sample.	Water-free.	Original sample.	Water-free.
OFFICIAL METHOD.				
E. B. Holland, Massachusetts	*Per cent.* 14.36	*Per cent.* 15.61	*Per cent.* 11.02	*Per cent.* 11.70
	14.79	.....	11.13	.....
	14.34	.....	10.69	.....
	14.34	.....	10.75	.....
	.....	.....	10.75	.....
	.....	.....	10.90	.....
H. J. Warner, U. S. Department of Agriculture	12.99	13.92	10.62	11.07
	12.96	.....	10.58	.....
	13.15	.....	10.43	.....
H. H. Hanson, Maine	14.09	15.05	10.95	11.43
C. D. Howard, West Virginia	14.25	15.34	11.08	11.68
	14.29	.....	11.02	.....
J. A. Bizzell, New York (Ithaca)	13.83	14.93	11.33	11.96
	13.95	.....	11.20	.....
L. H. Smith and E. M. East, Illinois	14.22	15.19	12.14	12.78
	14.33	.....	12.22	.....
	15.02	.....	12.03	.....
A. W. Bosworth, Rhode Island	9.57	[a]10.13	6.97	[a]7.42
	9.27	.....	7.12	.....
C. W. Mudge, New York (Geneva)	13.65	14.66	11.01	11.59
Average	.....	14.96	.....	11.74
KÖNIG METHOD.				
E. B. Holland, Massachusetts	19.97	22.35	11.51	12.49
	21.43	.....	11.69	.....
H. J. Warner, U. S. Department of Agriculture	17.20	18.44	11.75	12.20
	17.20	.....	11.48	.....
	17.34	.....	11.62	.....
H. H. Hanson, Maine	19.78	21.13	14.97	[a]15.64
C. D. Howard, West Virginia	18.06	19.08	11.98	12.27
	17.73	.....	11.52	.....
	17.45	.....	11.31	.....
J. A. Bizzell, New York (Ithaca)	15.58	16.54	11.76	12.50
	15.18	.....	11.80	.....
L. H. Smith and E. M. East, Illinois	17.49	18.37	13.70	13.18
	17.70	.....	12.27	.....
	17.51	.....	11.56	.....
A. W. Bosworth, Rhode Island	.....	.....	11.55	12.15
C. W. Mudge, New York (Geneva)	19.45	22.21	11.50	12.61
	21.90	.....	12.46	.....
Average	.....	19.73	.....	12.49
KÖNIG METHOD, MODIFIED.				
E. B. Holland, Massachusetts	11.70	12.73	9.97	10.82
	11.88	.....	10.13	.....
H. J. Warner, U. S. Department of Agriculture	11.03	11.88	9.55	9.96
	11.07	.....	9.52	.....
	11.26	.....	9.40	.....
J. A. Bizzell, New York (Ithaca)	11.44	12.36	10.39	10.94
	11.56	.....	10.22	.....
L. H. Smith and E. M. East, Illinois	11.66	12.17	10.24	11.24
	11.61	.....	11.09	.....
A. W. Bosworth, Rhode Island	14.48	[a]15.58	9.72	10.23
C. W. Mudge, New York (Geneva)	10.75	11.48	9.52	9.70
	10.62	.....	8.92	.....
Average	.....	12.12	.....	10.48

[a] Results omitted from average.

NOTES AND COMMENTS OF ANALYSTS.

E. B. Holland.—In the official method both filtrations were made through linen and the residue finally transferred to a platinum Gooch crucible and dried at 100°C.

With the König, and modified König, methods the acid solution was diluted with an equal volume of hot water and filtered through linen.

H. J. Warner.—The König method while extracting pentosans evidently leaves a large amount of nitrogenous matter unextracted. The modified König method seems to give the best results.

H. H. Hansen.—König's method did not give satisfactory results in any case. Difficulty was encountered in filtering. Both Gooch and linen filters were tried using suction pump and diluting the acid mixture with hot water. In some cases the filtration stopped entirely.

C. D. Howard.—In filtering, a thin pad of asbestos upon a 2-inch Hirsch funnel seemed to give the best results. Filtration was found to be very materially hastened by washing fiber back into a beaker once or twice, replacing any asbestos that may have been removed. Dilution of the hot mixture before filtering was found to be helpful in the case of wheat bran, but gave no advantage with distiller's grains. The washing with alcohol is tedious, and the extent to which it is carried appears to have a material influence upon results. The present official method seems preferable to the König, apparently giving results nearer the truth, besides being quicker and easier of operation.

J. A. Bizzell.—In the fiber determinations the last filtration was made on linen, the residue transferred to a platinum dish and evaporated to dryness. This method was found preferable to filtration on a Gooch. In the König method the mixture was diluted with alcohol and filter through linen. The filtration was very slow.

L. H. Smith and E. M. East.—When asbestos was mixed in the sample before boiling, and the filtration made on hardened filter paper in a 4-inch Buchner funnel, the acid mixture being diluted with 300 cc of hot water, filtration was obtained in three minutes. Other methods took from ten to twelve hours. In comparing the two methods, König and official, we have to consider besides the point of accuracy those of convenience and expense. In regard to convenience the relative length of time required for the operation is about the same. As to expense, the cost of reagents is greatly increased by the use of glycerol and ether in the König method. The results by the modified König method are all lower than by the official method, showing a further solvent action by the alkali.

A. W. Bosworth.—In the König, and modified König, methods there was great difficulty in filtering, requiring a long time and giving opportunity for error.

COMMENTS OF REFEREE.

As stated above, the official method for crude fiber does not remove the pentosans, and the König method fails to remove albuminoids, hence the two methods are in no way comparable. The additional treatment with alkali, as in the modified König method, gives us a fiber which is much purer, contains very little nitrogen, and is nearly free from pentosans. The following table is of interest as showing the nitrogen content of the fiber obtained by the three methods:

TABLE V.—*Nitrogen in crude fiber.*

Source.	Official method.	König method.	Modified König method.
	Per cent.	*Per cent.*	*Per cent.*
Distiller's grains	0.27	1.94	0.26
Wheat bran	.00	.195	.00

The results shown in the above table explain why the percentage of fiber obtained by the König method is so much higher than that secured by the official method. This is especially noticeable in the case of the distiller's grains, which carry 5.5 per cent of nitrogen, and your referee believes that the fiber from cottonseed-meal and all feeding materials high in protein, would also carry a high percentage of nitrogen.

In comparing the modified König method with the present official method there are several points to be taken into consideration as mentioned by Messrs. Smith and East in their comments on the König method. Convenience, expense, and, above all, accuracy, are factors which can not be ignored when we are considering any method of chemical analysis. As the modified König method now stands it is not quite so convenient as the official and more expensive, but the point which deserves especial attention is that of accuracy.

Table IV shows that distiller's grains yield nearly 3 per cent and bran 1.25 per cent more fiber when treated as in the official method than when submitted to the action of glycerol-acid mixture with subsequent treatment with alkali.

Your referee does not believe that this difference is due entirely to the presence of pentosans in the fiber from the official method, but that the acid in the presence of glycerol at a temperature of 131° to 133° C. exerts a hydrolytic action upon the fiber in the material. To throw some light upon this point, the effect of the glycerol-acid mixture on pure cellulose was noted. Practically pure cellulose was obtained by treating absorbent cotton, first with dilute sulphuric acid, then with dilute sodium hydrate, washing thoroughly and drying to constant weight in a steam oven at 98° C. The water-free residue was then treated by the König method with results as shown in the following table:

TABLE VI.—*Treatment of cellulose with glycerol-sulphuric acid.*

No.	Weight of charge (gram).	Amount recovered.	
		Gram.	Per cent.
1	0.2630	0.2305	87.6
2	.2772	.2431	87.7

Of course, if this solvent action takes place in the König method, as the above figures indicate, the modified method would offer no solution to the problem.

Your referee feels some reluctance in condemning the modified method until further evidence is produced corroborating the results shown in the above table. On the other hand, if it be proved conclusively that the acid mixture has no solvent action on the fiber, I believe the modified method can be made practicable by giving special attention to the details of manipulation.

RECOMMENDATIONS.

It is recommended that the referee and associate for 1904 conduct research work mainly along two lines:

(1) An inquiry into the true nature of the ether extract obtained after digesting the residue obtained by the official method of fat extraction, with pepsin.

(2) To prove or disprove that the glycerol-acid mixture of the modified König method has a hydrolytic action upon cellulose.

The SECRETARY. I have a paper on feeding materials from Mr. Browne, of the Louisiana station, which I am requested to submit to the association in his absence.

NOTES ON THE ANALYSIS OF FEEDING MATERIALS.

By C. A. Browne, Jr.

I.—Occlusion of Fat as a Source of Error in Analytical Work.

At the last convention of the Association of Official Agricultural Chemists,[a] the referee on cattle feeds called attention to a statement by Beger regarding the incomplete extraction of fat from certain materials high in protein unless these substances were first subjected to a digestion with pepsin solution. In the course of some feeding experiments recently conducted at the Louisiana Experiment Station upon rice bran and rice polish, an interesting and peculiar confirmation of the above statement was brought to light. With these feeds themselves no appreciative difference was found in the percentage of fat as determined by the ordinary official method and by the pepsin method. On the feces from the steers fed upon these products, however, very decided differences were obtained, the excess of fat by the pepsin method being in one instance over 4 per cent. A comparison of results is given in the following table:

Table I.—*Determination of fat in dried feces by official method and pepsin method.*

Sample of feces.	Time of extraction.	Fat.		Protein.	
		Official method.	Pepsin method.	Before pepsin digestion.	After pepsin digestion.
	Hours.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1. Rice bran	16	9.80	11.56	9.49	4.62
2. Rice polish	16	6.19	6.63	21.50	11.94
3. Rice polish	16	8.17	12.85	23.44	13.56
	24	8.94	.....	.....	.....
	32	10.00	.....	.....	.....

The extraction of the fat by the pepsin method was effected with ease and rapidity, whereas an extraction by the ordinary method, continued for thirty-two hours in one instance, left 3 per cent of fat unremoved. The difficulty was due no doubt to an occlusion of the fat by protein matter. This was further indicated by the fact that in some instances nearly 10 per cent of protein was removed from the dried feces by the pepsin digestion.

The error which may result from an incomplete extraction of fat, either in the food or feces, may seriously vitiate the calculation of digestion coefficients, as is seen from the following table:

Table II.—*Digestive coefficients of fat.*

Sample.	Calculated from results of—	
	Official method.	Pepsin method.
	Per cent.	*Per cent.*
1. Rice bran	59.8	52.0
2. Rice polish	82.5	81.2
3. Rice polish	78.2	66.0

[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. No. 73, Proceedings Nineteenth Annual Convention A. O. A. C., p. 148.

Erroneous determinations of fat may thus introduce a considerable error into tables of digestive coefficients, particularly in the case of feeds having a high content of both fat and protein.

A similar source of error in determining fat has been observed in the analysis of mixed feeds containing molasses, the occlusion in this instance being due, not to the protein constituents, but to the dried residue of sugars. With feeds containing molasses, it is recommended to wash 2 to 5 grams of the material on a filter with about 200 cc of cold water, then dry and extract with ether in the usual way.

II.—Determination of Acidity in Feeding Materials.

In the proceedings of the nineteenth annual convention of the association Gudeman describes a method for the determination of acidity in gluten feeds. This method, which consists in titrating an aqueous extract of the feed with standard alkali, gives only the water soluble acid, and is not always satisfactory when the total acidity of a feed is desired, this being particularly the case with feeding materials containing free fatty acids. The following process has been found very satisfactory when the total acidity of a feed is desired:

Twenty grams of the feed are transferred to a 100 cc flask. Eighty cc of 90 per cent alcohol are then added and the flask heated for 30 minutes at a temperature sufficient to keep the contents at a gentle boil. A condensing tube should be used to prevent the loss of alcohol. After cooling, the volume of the flask is made up with 90 per cent alcohol to 105 cc, allowing 5 cc for the volume of feed. After mixing thoroughly the alcoholic solution is poured through a dry filter and 50 cc of the clear filtrate (10 grams of feed) titrated with N/10 alkali. A comparison of the results obtained by direct alcoholic extraction with those calculated from the acid number of the fat in the case of a few samples of rice meal is given herewith:

Table III.—*Determination of acidity in feeds.*

Sample.	Acidity by alcoholic extraction.	Acidity calculated from acid number of fat.
	(cc N/10 NaOH per 10 grams feed.)	
1. Rice meal (fresh)	7.6	6.2
2. Rice meal (fresh)	15.5	12.2
3. Rice meal (old sample)	33.7	31.6

The results by alcoholic extraction are a little higher than those calculated from the acid number of the fat, indicating the presence of small amounts of other acid products besides the free fatty acids.

Mr. Wiley. I wish to call attention to a communication received from the international committee on the analysis of foods and feeding stuffs asking for the collaboration of the American chemists. This committee, which includes a member from this country, aims to secure, if possible, uniform methods of analysis for these materials. Two other committees of the same kind have been in operation for several years, namely, the committee on international methods of sugar analysis and a committee on international methods of fertilizer analysis. It is hoped that by this international collaboration the methods employed

throughout the world will practically become uniform. They are uniform in essence now but not in detail, and these committees are adopting and considering very carefully, sometimes adopting en bloc, with little or no change, the methods of this association, showing that our work is fully appreciated by these international committees. I happen to be a member of all three of these committees, and so keep in close touch with their work.

On the motion of Mr. Van Slyke the convention adjourned at 2.15 to attend the session of the Association of American Agricultural Colleges and Experiment Stations.

SECOND DAY.

FRIDAY—MORNING SESSION.

The convention was called to order by the president at 9.30 o'clock a. m., when the report of the referee on fats and oils was received.

REPORT ON FATS AND OILS.

By L. M. Tolman, *Associate Referee.*

The Hübl method for determining the iodin absorption of oils and fats is the official method of this association, but it has several faults which affect its accuracy. First, it rapidly loses strength if made up as the official method directs, and in a week or so is unfit for use; second, it is so slow in its reaction with some of the oils, such as linseed, that a serious error is brought about by the change in strength in the solution during the reaction. Wijs[a] shows how great this error might be, and the following table shows how much difference the time of titrating the blank makes in the iodin number.

Table I.—*Iodin number of linseed oil by Hübl method.*

Time of absorption.	Blank titrated at beginning.	Blank titrated at end.
Hours.		
2	173.74	.....
7	177.65	170.39
24	181.89	163.16

These figures show a decrease in the iodin number after seven hours, if the blank is titrated at the end of the determination. Wijs considers that the true iodin number in this case lies between 173.7 and 181.89. This change in strength can be greatly lessened by using purified absolute alcohol, but even under these conditions the solution soon becomes so weak that it is of no further use.

Another objection to the Hübl method is that practically each analyst employs a modification of his own, especially, as regards the time necessary for the solution to remain in contact with the oil; and, as is well known, very different results are obtained in this way on the same oils, which results have been collected as data obtained by the Hübl method. The following directions from various authorities as to the time needed for the Hübl test show how varied is the practice in this regard. Allen[b] recommends two hours. Our official method requires three hours.

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[a] Chem. Rev. Fett- u. Hartz-Ind., 1899, **6**: 6.

[b] Com. Org. Anal. **2**: 64, pt. 1. Fett- u. Hartz.

Lewkowitsch[a] recommends four hours. Wijs[b] showed that with linseed oil seven hours was necessary, and it is a very common practice among analysts to allow from 18 to 24 hours. These different practices can not fail to bring about diverse results.

The chief objection to changing the method is that it has been used for a great number of years and a great deal of valuable data on oils and fats has been obtained by its use. If a method can be suggested which will have permanence and speed and still give results which agree with the figures obtained by the Hübl method there seems to be every reason for discarding the old method.

Three solutions have been suggested as substitutes for the Hübl method which overcome to a great degree its faults, and a study of these methods has been the work of the referee this year. Cooperative work with chemists on the Wijs and Hanus methods has been done, while the bromin solution has been a subject of special study by the referee, as has also the excess of reagents necessary and the speed of reaction of the other solutions.

The keeping qualities of the Wijs and Hanus solutions have been thoroughly tested and found to be very satisfactory when the acetic acid is pure and of 99 per cent strength. The bromin solution has not been so thoroughly tested, but a solution of N/3 bromin in pure carbon tetrachlorid showed very little change in strength in 5 months, which would make it perfectly satisfactory on that score. Some peculiarities of the bromin solution will be noted later.

The cooperative work was limited to a comparison of the results obtained on various oils by the Hübl, Hanus, and Wijs methods, the object being to find what difference in the present limits on iodin numbers would be brought about by adopting either of the new methods. Six samples of oils, with the following directions, were sent out by the referee to a number of chemists interested, with the request that careful comparative work be done on these with both the Hanus and Wijs, and that figures be reported on such other fats and oils as might be available.

Directions for Iodin Work.

Hübl method.—Follow method as given in Bulletin No. 46 or No. 65. Allow excess of at least 50 per cent of the total iodin added.

Hanus method.—Dissolve 13.2 grams iodin in 1,000 cc glacial acetic acid (showing no reduction with bichromate and sulphuric acid); add enough bromin to double the halogen content determined by titration. Three cubic centimeters of bromin is about the proper amount. The iodin may be dissolved by the aid of heat, but the solution should be cold when bromin is added.

Wijs method.—Dissolve 13.2 grams iodin in acetic acid, same as used in Hanus solution, and run in current of dried and purified chlorin gas until the halogen content is nearly doubled; a slight excess of iodin should be allowed. The solution may also be made by dissolving 10 grams iodin trichlorid in glacial acetic acid and adding 10.8 grams of iodin.

These two solutions are used in the same manner as in the Hübl method except that an excess of 70 per cent of the iodin added is allowed; 10 cc of the potassium iodid solution is added instead of 20 cc, and the time of reaction is shortened to 15 minutes and 30 minutes, depending on whether a nondrying oil, a drying oil or a fat is tested. After the potassium iodid solution is added the mixture must be shaken thoroughly before adding the water.

In reporting results state details as to methods and manipulation.

References:

Hanus: Zeits. Nahr. Genussm., 1901, **4**: 913; J. Soc. Chem. Ind., 1902, p. 455.

Wijs: Berichte, 1898, p. 752; J. Soc. Chem. Ind., 1902, p. 455.

As a result of the remarkable interest shown, enough figures have been reported on many kinds of fats and oils to give a very satisfactory basis of comparison for the three methods. These results have been collected in the following tables. Table II was prepared from results received on the samples sent out by the associate referee.

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[a] Chem. Analysis of Oils, Fats, and Waxes, 1899, p. 173.

[b] Chem. Rev. Fett- u. Hartz-Ind., 1899, **6**: 7.

TABLE II.—*Results on official samples of oils.*

Kind of oil.	Hübl number.	Hanus number (30 minutes).	Wijs number (30 minutes).	Difference Hanus and Hübl numbers.	Difference Wijs and Hübl numbers.	Analyst.
Olive	89.8	90.0	91.4	+ 0.2	+ 1.6	Tolman and Munson.
Do	89.0	89.6	90.5	+ .6	+ 1.5	Blasdale.
Do	88.3	90.2	92.2	+ 1.9	+ 3.9	Van Wormer.
Do	87.0	88.2	90.8	+ 1.2	+ 3.8	Adams.
Do	89.5	90.2	90.2	+ .7	+ .7	Lane.
Rape	101.3	105.2	105.7	+ 3.9	+ 4.4	Tolman and Munson.
Do	100.6	103.2	104.7	+ 2.6	+ 4.1	Blasdale.
Do	99.9	104.3	103.1	+ 4.4	+ 3.2	Van Wormer.
Do	102.0	101.8	105.2	− .2	+ 3.2	Adams.
Do	101.3	101.5	105.7	+ .2	+ 4.4	Lane.
Mustard	113.6	116.8	118.2	+ 3.8	+ 5.2	Tolman and Munson.
Do	111.7	115.2	116.9	+ 3.5	+ 5.2	Blasdale.
Do	114.1	116.2	118.4	+ 2.1	+ 4.3	Van Wormer.
Do	112.4	113.5	117.9	+ 1.1	+ 5.5	Adams.
Do	112.4	113.0	116.9	+ .6	+ 4.5	Lane.
Maize	123.3	126.0	129.2	+ 2.7	+ 5.9	Tolman and Munson.
Do	114.1	123.4	125.9	+ 9.3	+11.8	Blasdale.
Do	118.8	123.1	126.8	+ 4.3	+ 8.0	Van Wormer.
Do	120.9	122.0	124.8	+ 1.1	+ 3.9	Adams.
Do	121.9	121.7	125.7	− .2	+ 3.8	Lane.
Poppy	134.9	138.4	139.1	+ 3.5	+ 4.2	Tolman and Munson.
Do	133.9	135.1	136.7	+ 1.2	+ 2.8	Blasdale.
Do	135.1	134.9	134.9	− .2	− .2	Van Wormer.
Do	132.8	132.4	136.0	− .4	+ 3.2	Adams.
Do	138.2	136.4	138.5	− 1.8	− .3	Woodman.
Do	132.0	133.3	135.8	+ 1.3	+ 3.8	Lane.
Linseed	.....	183.7	188.7	.....	.....	Tolman and Munson.
Do	167.7	185.1	188.4	+17.4	+20.7	Blasdale.
Do	166.7	186.1	188.6	+19.4	+21.9	Van Wormer.
Do	173.0	180.9	187.2	+ 7.9	+14.2	Adams.
Do	165.1	186.3	189.2	+21.2	+24.1	Lane.

All these oils have a comparatively high iodin number and the agreement between the results obtained by the different methods is not so close as is found with the fats and oils of a low iodin number. The Hübl method, especially on the oils with high iodin numbers, shows many discrepancies between the results of the various analysts. The Wijs method gave the best results; especially is this true with linseed oil where the maximum difference between the results of the five analysts was 2.00 numbers. The Hanus method also gives good results, which are uniformly lower than those of the Wijs method, making a closer agreement with figures at present obtained by the Hübl method. This agreement, however, is not of great value on these oils, especially the rape, mustard, maize, poppy, and linseed oils, as the published data give such wide limits.

These wide limits are doubtless largely due to the varying methods and modifications of the Hübl method used in the determination. The Hübl solutions used in this work were not more than 24 hours old, and the results show this wide variation. If they had varied in age from one week to three weeks, probably a very much wider variation would have been found. This point is illustrated in the table following, taken from Benedikt and Ulzer, page 205.

TABLE III.—*Results obtained with Hübl solutions of varying ages.*

Age of Hübl solution.	Excess of iodin.	Iodin number of linseed oil.	Equivalent of 25 cc of solution in N/10 thio.
Days.	*Per cent.*		*Cc.*
1	62.5	201.0	47.11
2	64.0	198.5	45.7
4	65.0	196.7	44.25
11	67.0	191.0	38.89
26	68.0	185.9	31.81

These figures show a difference of 5 numbers with the same solution. It is on oils of this kind that the new solutions will give much more satisfactory results.

Table III contains the results obtained by G. E. Patrick on 51 butters and 14 oleomargarins by the Hübl and Hanus methods. In every case he obtained slightly higher results with the Hanus method than with the Hübl. The average on 51 samples of butter was 1.01 numbers, and the maximum was 1.97 numbers. On the 14 samples of oleomargarin the average was 1.14 numbers higher and maximum of 1.61 numbers. These results were on fresh and rancid products, the results showing no difference.

TABLE IV.—*Comparison of Hübl and Hanus methods on butter and oleomargarin (G. E. Patrick).*

BUTTERS.

Laboratory No.	Hübl.		Hanus.		Difference between Hanus and Hübl numbers.	Remarks.
	No.	Time.	No.	Time.		
		Hrs.		*Min.*		
139	33.68	4	34.59	30	+0.91	
140	28.98	4	29.42	75	+ .44	
141	31.39	4	32.00	60	− .61	
142	30.20	4	30.47	30	+ .27	
143	30.57	4	30.82	60	+ .25	
144	36.24	4	37.06	60	+ .82	
145	35.27	4	36.38	30	+1.11	
146	34.50	4	35.62	30	+1.12	
147	32.16	4	33.15	30	+ .99	
927	29.24	4	29.84	30	+ .64	
928	35.97	4	36.56	30	+ .58	
151	29.51	3	31.25	30	+1.74	
153	35.62	3	37.59	30	+1.97	
27	28.11	3	29.99	30	+1.88	Very rancid.
35	31.59	3	33.28	30	+1.69	
32	33.38	3	34.67	30	+1.29	
75	26.90	3	27.96	30	+1.06	
78	36.79	3	38.28	30	+1.49	
864	35.04	3	36.95	30	+1.91	
871	29.48	3	31.24	30	+1.76	
872	31.41	3	32.71	30	+1.30	
1089	43.08	4	44.59	30	+1.51	Fresh.
1102	31.38	4	32.24	30	+ .86	
1108	33.66	4	34.14	30	+ .48	

TABLE IV.—*Comparison of Hübl and Hanus methods on butter and oleomargarin (G. E. Patrick)*—Continued.

BUTTERS—Continued.

Laboratory No.	Hübl.		Hanus.		Difference between Hanus and Hübl numbers.	Remarks.
	No.	Time.	No.	Time.		
		Hrs.		*Min.*		
73	34.19	4	34.76	30	+ .57	
82	35.52	4	36.23	30	+ .71	
85	35.53	4	36.38	30	+ .85	
.....	41.05	4	42.35	30	+1.30	Very rancid.
1141	39.75	4	41.27	30	+1.52	
1089	42.86	4	44.27	30	+1.41	
1090	43.03	4	44.44	30	+1.41	
81	39.65	3½	40.67	30	+1.02	Rancid.
84	34.45	3½	34.97	30	+ .52	
871	27.85	3½	28.31	30	+ .46	
872	30.24	3½	30.58	30	+ .34	
151	28.54	3½	29.03	30	+ .49	
153	33.93	3½	34.69	30	+ .76	
74	34.98	4	36.12	30	+1.14	
76	35.76	4	36.77	30	+1.01	
77	36.98	4	38.28	30	+1.30	
79	35.90	4	36.77	30	+ .87	Do.
80	39.48	4	40.92	30	+1.44	
83	32.70	4	33.66	30	+ .96	
1157	39.77	4	40.04	30	+ .27	
1159	38.73	4	39.64	30	+ .91	
1160	41.30	4	42.54	30	+1.24	
1161	37.28	4	37.94	30	+ .66	
1162	35.69	4	36.65	30	+ .96	Fresh.
1163	32.52	4	33.43	30	+ .91	
1156	37.72	4	38.52	30	+ .80	
1158	39.52	4	40.27	30	+ .75	
					+1.01	Average.
					+1.97	Maximum.

OLEOMARGARIN.

49	46.38	3	47.99	60	+1.61	Very rancid.
37	41.58	3	43.12	45	+1.54	Do.
XXX	49.39	3	50.69	30	+1.30	Do.
118	59.93	3	61.01	30	+1.08	Do.
1010	54.74	3	55.70	30	+ .96	Do.
21805	46.45	3	47.62	30	+1.17	
21920	66.17	3	66.65	30	+ .48	
21923	65.29	3	66.44	30	+1.15	
21925	52.94	3	53.84	30	+ .90	Two years old.
22287	68.91	3	69.18	30	+ .27	
22548	50.68	3	52.06	30	+1.38	
23375	46.64	3	48.05	30	+1.41	
995	72.39	3	73.90	30	+1.51	Four months old.
1073	72.24	3	73.51	30	+1.27	Three months old.
					+1.14	Average.
					+1.61	Maximum.

TABLE V.—*Results on miscellaneous oils and fats.*

Kind of oil or fat.	Hübl number (3 hours).	Hanus number (30 minutes).	Wijs number (30 minutes).	Difference between Hanus and Hübl numbers.	Difference between Wijs and Hübl numbers.	Analyst.
Cocoanut	8.93	8.60	9.05	− 0.33	+ 0.12	Tolman and Munson.
Do	8.31	8.12	.....	− .19	.....	Blasdale.
Butter	35.3	35.3	36.2	.00	+ .90	Tolman and Munson.
Do	34.8	35.4	35.9	+ .60	+ 1.10	Do.
Do	28.96	29.91	30.48	+ .95	+ 1.52	Woodman.
Do	29.90	30.0	30.60	+ .10	+ .70	Van Wormer.
Do	35.5	35.4	.....	− .10	.....	Adams.
Do	41.0	41.4	.....	+ .40	.....	Do.
Do	42.1	41.9	.....	− .20	.....	Do.
Do	40.5	41.0	.....	+ .50	.....	Do.
Oleomargarin	42.6	43.3	43.5	+ .70	+ .90	Tolman and Munson.
Do	53.6	52.3	53.5	− 1.30	− .10	Do.
Do	52.8	52.2	53.7	− .60	+ .90	Do.
Do	52.5	52.0	52.9	− .50	+ .40	Do.
Do	66.3	64.8	66.0	− .30	− 1.50	Do.
Do	64.33	65.7	66.4	+ 1.40	+ 2.10	Woodman.
Do	52.00	52.0	52.2	.00	+ .20	Van Wormer.
Do	66.1	67.8	67.5	+ 1.70	+ 1.40	Do.
Do	47.7	48.5	.....	+ .80	.....	Adams.
Do	46.6	47.6	.....	+ 1.00	.....	Do.
Do	49.8	50.5	.....	+ .70	.....	Do.
Do	46.4	48.0	.....	+ 1.60	.....	Do.
Do	50.2	51.8	.....	+ 1.60	.....	Do.
Do	48.8	48.9	.....	+ .10	.....	Do.
Do	47.3	48.2	.....	+ .90	.....	Do.
Olive	82.55	84.6	.....	+ 2.1	.....	Woodman.
Cotton seed	103.8	105.2	105.3	+ 1.40	+ 1.50	Tolman and Munson.
Do	106.2	107.8	107.3	+ 1.60	+ 1.10	Do.
Do	104.8	106.7	106.2	+ 1.90	+ 1.40	Do.
Do	111.90	111.2	113.4	− .70	+ 1.50	Woodman.
Do	95.84	96.5	99.9	+ .66	+ 4.1	Van Wormer.
Do	94.09	97.47	96.97	+ 3.38	+ 2.88	Blasdale.
Do	108.2	106.6	.....	− 1.6	.....	Adams.
Do	106.6	104.7	.....	− 1.9	.....	Do.
Do	105.6	103.1	.....	− 2.5	.....	Do.
Peanut	96.3	97.4	99.0	+ 1.10	+ 2.70	Tolman and Munson.
Do	94.5	94.1	95.2	− .40	+ .70	Do.
Do	107.7	107.7	109.5	.00	+ 1.80	Do.
Mustard	110.4	115.5	118.5	+ 5.10	+ 8.10	Do.
Do	113.0	116.8	118.2	+ 3.80	+ 5.20	Do.
Do	98.4	103.8	104.3	+ 5.40	+ 5.90	Do.
Do	103.5	110.2	112.5	+ 6.70	+ 9.00	Do.
Do	106.4	114.8	117.3	+ 8.40	+10.90	Do.
Rape	101.3	105.2	105.7	+ 3.90	+ 4.40	Do.
Do	100.2	102.8	104.1	+ 2.60	+ 3.90	Do.
Sunflower	106.4	107.2	109.2	+ .80	+ 2.80	Do.
Sesame	106.4	106.5	107.0	+ .10	+ .60	Do.
Do	95.9	103.5	100.8	+ 7.6	+ 4.9	Blasdale.
Maize	119.0	120.2	123.2	+ 1.20	+ 4.20	Tolman and Munson.
Do	119.0	119.6	122.2	+ .60	+ 3.20	Do.
Poppy	133.4	132.9	135.2	− .50	+ 1.80	Do.
Linseed	169.8	184.5	186.5	+14.70	+16.70	Do.

Mr. Patrick makes the following comment:

The Hanus solution has two strong points in its favor, namely, good keeping qualities and rapidity of action on the fats. The latter feature is important not alone because it expedites work, but also because it very largely obviates the errors due to expansion of liquids, provided blanks be run at rather frequent intervals throughout the day's work. The advantages of the Hanus solution are so marked that I have adopted it for regular use.

Mr. Patrick's results and those reported by the other analysts on butter and oleomargarin indicate that the Hanus method could be substituted for the Hübl in the examination of butter and similar products without any appreciable change in the limits at present held on these products. The same may also be said of the Wijs reagent, although in nearly every case the Wijs shows slightly higher results on these fats than the Hanus.

Table V contains the results on miscellaneous oils and fats sent in by the various chemists.

Table VI contains the results on 11 samples of pure olive oil prepared by G. E. Colby, of the University of California, and sent by him to W. C. Blasdale and the referee. These results show that the Hanus solution gave the best comparative results, there being a smaller average difference among results obtained by that method than with others, and a lower maximum difference, the Hübl method showing the greatest difference and the Wijs the next. This would indicate that the Hanus and Wijs methods had a tendency to give more uniform results than the Hübl, which is certainly a strong point in their favor. This fact was also noted in Table II giving results on the official samples. It is also evident from Table VI that the Wijs solution gives slightly higher results on olive oils. This confirms the referee's previous work [a] in which the average difference on 36 samples was +1.2 numbers.

TABLE VI.—*Pure olive oils.*

Number.	Hübl number.			Hanus number.			Wijs number.			Maximum difference.		
	Tolman.	Blasdale.	Colby.	Tolman.	Blasdale.	Colby.	Tolman.	Blasdale.	Colby.	Hübl.	Hanus.	Wijs.
1	83.7	82.44	83.1	83.7	83.60	83.7	85.0	83.44	83.7	1.3	0.1	1.6
2	83.7	81.69	83.7	84.0	83.24	83.0	85.9	83.73	84.2	2.0	1.0	2.2
3	79.9	77.70	79.7	79.1	79.51	80.5	81.3	79.22	81.3	2.2	1.4	2.1
4	86.1	85.40	85.0	85.6	86.65	85.5	86.6	85.57	87.9	1.1	1.1	2.4
5	83.2	83.53	82.9	83.0	84.24	83.9	84.4	83.13	83.7	.6	1.2	1.3
6	84.6	82.90	83.8	84.6	85.64	85.5	85.4	83.84	86.0	1.7	1.0	2.2
7	90.6	87.37	90.0	89.8	89.46	90.5	91.5	90.62	91.4	3.3	1.1	.9
8	85.4	83.42	84.8	85.1	84.88	86.7	86.7	85.03	85.3	2.0	1.9	1.7
9	84.8	82.69	85.1	84.1	84.18	85.0	85.9	84.16	86.9	2.4	.9	2.8
10	86.1	84.47	85.8	86.7	85.75	87.6	87.8	86.65	87.0	1.7	1.9	1.2
11	84.4	84.72	83.0	84.1	85.49	84.0	85.1	84.66	84.6	1.7	1.4	.5
Average										1.8	1.2	1.7
Maximum										3.3	1.9	2.8

The Hanus figures, obtained by the different analysts, do not have quite the same close agreement. The referee has obtained very close agreement between Hanus and Hübl. On 36 samples previously reported the average difference was 0.59, and on the 11 samples of Table VI an average of 0.24. Blasdale gets a slightly wider difference of 1.4 numbers, while Colby gets 0.89. The average of all would give less than 1. These results seem to show that these solutions are satisfactory substitutes.

[a] Tolman and Munson, J. Amer. Chem. Soc., 1903, **25**: 244.

TABLE VII.—*Cooperative work by N. J. Lane.*

Substance.	Iodin values of straight oil.								Iodin values of liquid acids of oils.						Remarks.
	Hübl method.		Hanus method.			Wijs method.			Per cent of liquid acid.	Hübl method 14 hours.	Hanus method.		Wijs method.		
	3 hours.	6 hours.	15 minutes.	30 minutes.	60 minutes.	15 minutes.	30 minutes.	60 minutes.					1 hour.	2 hours.	
Cotton-seed oil	104.9			104.5	106.4		107.4	109.2	75.58	136.3	1 hour. 139.7				"Off" oil.
Do	108.2			108.1	108.8		112.5	112.2	77.18	139.0	1 hour. 135.3	3 hrs. 15 min. 152.4			"White" oil.
Do	111.3			109.2	110.0		113.7	111.6	78.79	140.2	1 hour. 140.7	2 hours. 147.1	145.1	143.7	White oil Hübl value is average of several determinations.
Steam lard	65.97		65.94	65.19		72.11	72.93		60.64	91.24	1 hr. 20 min. 94.54	2 hours. 89.71	98.83	101.7	Liq. acids by petroleum ether; per cent too low.
Tallow	51.74		53.68	52.84		55.27	53.89		58.79	85.39	1 hour. 84.28	2 hrs. 15 min. 84.74			Hanus 3 hrs. 10 min. 86.98.
Linseed oil	165.1	181.4		186.3	185.7		189.2	185.9							A. O. A. C. sample.
Olive oil	89.50		90.22	90.16		90.10	90.20								A. O. A. C. sample Aug. 25, 1903.
Maize oil	121.9			121.7	123.2		125.7	125.4							A. O. A. C. sample Aug. 27, 1903.
Mustard-seed oil	112.1			112.1	113.0		116.9	116.3							A. O. A. C. sample Aug. 28, 1903.
Poppy oil	132.0			133.3	134.9		135.8	136.8							A. O. A. C. sample Aug. 28, 1903
Rape	101.3			101.5	101.5		105.8	105.6							A. O. A. C. sample Oct. 17, 1903
Cotton-seed oil and tallow			86.06						68.42	118.0	1 hour. 126.5				
Do			60.27						53.53	103.9	1 hour. 105.2				

Table VII contains the results reported by N. J. Lane, and includes some results on the iodin numbers of the liquid fatty acids of the various oils and fats. He makes the following comment in regard to the merits of the methods:

I think the Hanus is so far ahead that it requires no comment as to its use on the straight oils. The differences between it and the Hübl are so slight as to be negligible. Different samples of the same oil vary more than the results by these two methods on the same sample, and therefore it could readily be substituted for the Hübl without any inconvenience or change of standards.

Regarding the action of the Hanus solution on the liquid acids, the results do not agree very well, either among themselves or with the Hübl, but this may be a matter of time of action. For some reason liquid acids require more time for complete absorption of iodin than the straight oil and it is useless to attempt to get the Hübl value in less than 12 to 14 hours. The difference between the Hübl and the Wijs methods seems to render the Wijs impracticable for general use without a revision of all standards. It does not possess any advantage over the Hanus, and the solution is far more troublesome to prepare.

Comments by Cooperating Analysts.

W. C. Blasdale.—As to the relative merits of the three processes I think I should, on the whole, prefer the Hanus. The manipulation is quite as satisfactory as the Hübl and the shorter time required for a determination is a very decided advantage as well as the greater permanence of the solution.

A. G. Woodman.—I think, on the whole, I prefer the Hanus method to the Wijs for convenience in preparation of the solution and in use. Either is far preferable to the Hübl. I suggest the following methods for dissolving the iodin and weighing the fat:

HANUS SOLUTION.

Dissolve C. P. iodin in glacial acetic acid. Place the iodin in a flask immersed in hot water and add the acid in small amounts with frequent shaking and decanting until solution is complete. To the cold solution add the required amount of bromin.

WEIGHING SAMPLES.

Weigh out the oil in a small beaker containing also a short piece of glass tubing drawn out like a burette tip. To the upper end of this attach a small bit of rubber tubing containing a glass bead to close the end. Weigh the whole system, then by compressing and releasing the rubber tube draw a quantity of oil partly up the glass tube, transfer the required number of drops to the bottle and weigh the system again. Weigh the solid fats at such a temperature that they are liquid and homogeneous. Calculate the amount taken in each case so that about 70 per cent of the iodin is in excess.

A. B. Adams.—In their workings the Hanus and the Wijs methods are very similar, but of the two we prefer the Hanus, for the following reasons:

1. In making up the solutions, bromin is more easily obtained than iodin trichlorid.
2. The figures obtained in the Hanus are very nearly the same as those given by the official method (Hübl).

Of the three, Hanus, Wijs, and Hübl, we prefer the Hanus, for the following reasons:

1. Length of time required to make up the reagent—Hanus being ready immediately, the Hübl not until it has stood 12 hours.
2. The Hübl solution weakens rapidly, the Hanus very slowly.
3. With the Hanus method nearly all oils require only about 30 minutes for complete absorption, while with the Hübl 3 hours is not sufficient.
4. In agreement of duplicates the Hanus method is far superior; in fact, there is no comparison between the two.

There seems to be every reason in favor of the Hanus method. In our work the oleo is allowed to stand 30 minutes, as we think this gives safer figures in all cases.

Linseed oil should be allowed to stand 1 hour. In short, 30 minutes for oils or fats with a figure below 150, with a figure over that, 1 hour should be long enough.

R. E. Doolittle.—The only difficulty experienced with these methods was the weighing out of the trichlorid. This could be obtained in 1-oz. bottles, or even ½-oz., and the whole bottle used, which would do away with the difficulty.

The remainder of the paper is a report of work done by the referee in the Bureau of Chemistry:

THE WIJS SOLUTION.

Wijs[a] directs that there be an excess of 70 per cent of the iodin added in the determination in order to insure complete and rapid absorption, but the results on linseed oil given in Table VIII indicate that such a large excess is unnecessary. In each of the determinations 0.1500 gram of linseed oil was used and the solution was allowed to stand 30 minutes in contact with oil.

TABLE VIII.—*Iodin absorption of linseed oil with varying excess.*

Wijs solution.	Value in N/10 iodin.	Amount of iodin absorbed in N/10 iodin.	Iodin number.	Excess of iodin.
cc	*cc*	*cc*		*Per cent.*
25	61.28	23.16	193.4	62
20	49.35	23.13	193.7	53
15	37.08	23.13	193.7	37

These figures show that with an excess of only 37 per cent the same results were obtained in 30 minutes as with an excess of 62 per cent. Linseed oil was used on account of its high iodin number and because it takes longer to obtain complete absorption with it than with oils of a lower iodin value. This smaller excess effects a great saving in solution and makes titrations more rapid. This smaller excess does not greatly affect the speed of reaction, as is shown in Table IX.

TABLE IX.—*Effect of excess of Wijs solution on speed of reaction.*

Time.	Iodin numbers.	
	Excess of iodin 62 per cent.	Excess of iodin 37 per cent.
Minutes.		
1	191.1	189.0
5	191.9	191.2
10	191.1	191.4
15	191.7	191.4
30	192.3	191.4
60	193.1	.....

These results show that the reaction between the Wijs reagent and the oil is very rapid, even with only 37 per cent excess.

Table X shows that when the determinations are made in the light the iodin number seems to increase up to a definite point where it remains constant for a short time, then gradually increases up to 4 hours with no tendency to remain constant. In the dark the iodin number remained constant from 1 to 2 hours.

[a] Zeit. Nahr. Genussm., 1902, 5: 499.

TABLE X.—*Iodin number of linseed oil by Wijs solution.*

Time.	Iodin numbers.	
	In light.	In dark.
Minutes.		
1	192.4	186.1
10	193.1	192.6
30	193.1	192.9
60	194.1	193.5
120	194.7	193.4
240	195.7	.....

TABLE XI.—*Effect of time and light on Wijs and Hanus solutions.*

Time.	Iodin numbers.	
	In light.	In dark.
WIJS.		
30 minutes.	191.9	192.1
5 hours	194.0	193.0
HANUS.		
30 minutes.	189.1	189.2
5 hours	191.9	192.1

It will be noticed from these results that on the Wijs solution, standing 30 minutes, the light has no effect; the same may be said of the Hanus. In the 5-hour test two effects are noted in the Wijs, namely, there is a very slight increase in the iodin number over the figures obtained in 30 minutes, and the results obtained in the light are slightly greater than those obtained in the dark. With the Hanus solution there is a slight increase in 5 hours over 30 minutes, but no difference in light or dark. This would indicate that there it is not necessary to stand either the Wijs or Hanus solution in a dark cupboard when 30 minutes are allowed for the reaction.

This increase of absorption on long standing is probably due to secondary reactions as is shown in the results with iodin chlorid in carbon tetrachlorid given in Table XI. Upon standing longer than thirty minutes substitution takes place and longer standing shows a remarkable, though gradual, change in the results.

TABLE XI.—*Iodin number by iodin chlorid in carbon tetrachlorid.*

Time.	Total.	Addition.	Substitution.
Hours.			
5	210.8	184.9	12.9 in light.
5	204.8	190.9	6.9 in dark.
1	198.6	194.2	2.2 in light.
½	195.2	194.6	.80 in light.

These figures show that the addition apparently decreases with time after 1 hour, with a gradual increase in the total amount of iodin entering into combination. This indicates that with iodin chlorid in carbon tetrachlorid, standing longer than 30 minutes is no advantage and longer than 1 hour gives entirely unreliable figures. It

seems probable that the same thing will be shown with iodin chlorid in acetic acid although to a much less extent, and that long standing gives untrue results. With iodin bromid in carbon tetrachlorid the secondary reactions are much weaker, but the gradual increase up to 18 hours' standing with Hanus solution is doubtless due to the same cause. This indicates that there is a maximum as well as a minimum time which should be allowed for the reaction, and for the Wijs solution this limit is between 15 minutes and 1 hour, the advantage being with the shorter time rather than the longer, 30 minutes being satisfactory for either solution.

The Hanus Solution.

Hanus[a] also advises an excess of 70 per cent in order to insure complete absorption, and the results given in Table XII show that nearly this large excess is necessary to obtain complete absorption on linseed oil in 30 minutes.

Table XII.—*Iodin number of linseed oil with varying excess of iodin.*

Wijs solution.	Value in N/10 iodin.	Amount of iodin absorbed in N/10 iodin.	Iodin number.	Excess of iodin.
cc	*cc*	*cc*		*Per cent.*
25	54.76	23.26	196.3	57
20	44.08	22.73	191.9	48
15	33.06	21.86	184.5	34

These figures, if compared with Table VIII, show how much slower in reaction the Hanus solution is than the Wijs, and how much greater excess is needed to produce the same results. Table XIII also brings out the slowness of the Hanus solution as compared with the Wijs.

Table XIII.—*Iodin numbers by Hanus solution in varying times.*

Time.	Iodin No. 1. In light.	Iodin No. 2. In light.	Iodin No. 3. In dark.	Iodin No. 4. In dark.	Iodin No. 4. In light.
Minutes.					
1	164.8	164.6	166.9	.....	.....
5	178.9	.....	.....	.....	.....
10	181.3	182.2	187.7	.....	.....
15	184.6	186.7	.....	.....	.....
30	185.6	187.5	191.0	189.2	189.1
Hours.					
1	185.6	187.5	191.3	.....	.....
2	.....	188.5	191.0	.....	.....
4	.....	189.5	.....	.....	.....
5	.....	.....	.....	192.1	191.9
18	.....	193.3	.....	.....	.....

These results on samples of linseed oil by different analysts show that it takes 30 minutes by the Hanus solution to reach the constant stage which is reached by the Wijs in 10 minutes. The absorption is constant from 30 minutes to 1 hour and then begins the gradual rise, as was the case with the Wijs, and the same explanation is suggested. The Hanus is less affected by light than the other solutions.

[a] Zeit. Nahr. Genussm., 1901, 4: 913.

Bromin Solution and Carbon Tetrachlorid as a Solvent in the Wijs and Hanus Methods.

In connection with the work of testing the Hanus and Wijs solutions, it was thought well to test the bromin solution proposed by McIlhiney and also the substitution of carbon tetrachlorid as a solvent for acetic acid in the Hanus and Wijs methods. The advantage of this solvent is that it is neutral and permits of an estimation of the substitution which takes place in the reaction between the oil and the halogen. It was thought that if a change was to be made in our present method and any information of added value could be obtained by the use of a solvent of this nature, it would be well to adopt at this time the best method obtainable.

Solutions of iodin chlorid, iodin bromid, and bromin in carbon tetrachlorid were prepared, using the same strength as in the Wijs and Hanus methods and approximately N/3 bromin as directed by McIlhiney. A solution in carbon tetrachlorid of linseed oil of known purity was prepared and used in most of the experiments. The actual amount of oil used in each test was affected to a very slight degree by changes in temperature. As a rule, a fresh solution was made up for each set of tests and only very slight variations were found in the iodin numbers obtained by the Wijs and Hanus solutions in acetic acid at any time. In each determination 0.1500 of a gram of oil was used, which made it possible to regulate the excess of reagent and other conditions very exactly.

With these solutions several questions were to be decided:

1. How do the results obtained compare with those given by other solutions, especially the Hübl?
2. How long a time is required to obtain maximum figures?
3. Does water affect the amount of substitution?
4. How does light affect the reaction between oil and the halogen?
5. Does age of solution affect the results?

At this point it might be well to consider what results would be obtained if the acid formed in the Hübl reaction is estimated as substitution and the addition figure calculated from these figures. The formation of acid in the Hübl reaction has long been known and many suggestions have been made as to its cause; but the reaction has not as yet been satisfactorily explained, although it has been proved that it is not due to substitution.[a]

In Table XIV are collected the results on a linseed oil by the Hübl method, the acid formed being determined by addition of potassium iodate.

Table XIV.—*Results on linseed oil by the Hübl method, with addition of potassium iodate.*

Time of reaction.	Hübl number.	Addition.	Substitution.
Hours.			
½	161.9	124.7	18.6
1	165.0	127.4	18.8
2	170.5	130.9	19.3
3	175.1	135.5	19.3
5	180.0	146.4	16.8
24	181.0	153.2	13.9

The amount of acid remains practically the same, regardless of the time of reaction, the most satisfactory explanation being that there is a decomposition of the addition product formed in the reaction and the formation of an acid due largely to the presence of water. The occurrence of this decomposition can be easily proved by adding

[a] Ingle, J. Soc. Chem. Ind., 1902, p. 587.

potassium iodate after titrating the excess of free iodin and noting the rapid formation of free iodin on standing, which takes place so quickly that the determination of substitution or acid must be made with great rapidity or very different results will be obtained. These results are given as being suggestive in connection with those to be discussed later. The bromin solution will be considered first, the figures being given in Tables XV and XVI:

TABLE XV.—*Iodin numbers obtained by three different methods.*

Time.	Wijs method.				Hanus method.				Bromin method (in light).		
	Acetic acid.	Carbon tetrachlorid.			Acetic acid.	Carbon tetrachlorid.			Total.	Addition.	Substitution.
		Total.	Addition.	Substitution.		Total.	Addition.	Substitution.			
Minutes.											
1	186.1	.....	.....	.....	166.9	164.0	164.0	0.0	156.3	156.3	0.0
2	.....	192.8	192.8	0.0	.....	.....	.....	.....	.....	.....	.....
10	192.6	193.6	193.6	0.0	187.7	182.5	182.5	0.0	.....	.....	.....
15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
30	192.9	195.2	194.6	.80	191.1	188.6	188.6	0.0	193.2	193.2	0.0
60	193.5	198.6	194.2	2.20	191.3	189.7	189.7	0.0	197.0	197.0	0.0
120	193.4	.....	.....	.....	191.0	.....	.....	.....	196.7	194.2	1.27

TABLE XVI.—*Iodin numbers obtained by varying time of reaction in carbon tetrachlorid solution.*

Solution.	1 minute.			10 minutes.			30 minutes.		
	Total.	Addition.	Substitution.	Total.	Addition.	Substitution.	Total.	Addition.	Substitution.
Bromin	156.0	156.0	0.0	.....	.....	.....	193.2	193.2	0.0
Do	156.4	156.3	0.0	186.3	186.3	0.0	194.0	194.0	0.0
ICl in CCl_4	192.8	192.8	0.0	193.6	193.6	0.0	196.2	194.6	0.80
IBr in CCl_4	164.2	164.2	0.0	182.5	182.5	0.0	188.6	188.6	0.0

Solution.	60 minutes.			120 minutes.		
	Total.	Addition.	Substitution.	Total.	Addition.	Substitution.
Bromin	197.4	194.4	0.0	196.6	194.2	1.27
Do	198.2	198.0	0.0	203.8	202.0	.93
ICl in CCl_4	198.6	194.2	2.2	.....	.....	.....
IBr in CCl_4	189.7	189.7	0.0	.....	.....	.....

Table XV gives a comparison of the values obtained by the different methods on a sample of oil giving a Hübl number of 180. The solutions were all freshly prepared and C. P. carbon tetrachlorid, perfectly dry, was used in making them up. The results obtained show a very satisfactory agreement. The substitution figures are very small in all the solutions and appeared only after long standing in the Wijs and bromin solutions, being largest with the Wijs. The maximum addition figure was not reached with any of the solutions in less than one hour, as is shown in Table XVI. The results with the bromin solution were so different from those obtained on the same oil with other bromin solutions that the cause was sought with considerable interest. The relative speed of these solutions is about the same as with acetic acid as the solvent, as might be expected. The Wijs reagent is by far the most active, as shown in the other cases.

First a little water was added to the solution of oil in carbon tetrachlorid; then the fresh bromin solution was added and allowed to stand in the light for 1 hour and no substitution was shown, but when the water was added to the oil solution in carbon tetrachlorid and thoroughly shaken so that the water was taken up by the carbon tetrachlorid, the following results were obtained, the other conditions being the same:

TABLE XVII.—*Bromin absorption with solution of oil in 1 hour.*

Oil solution.	Total.	Addition.	Substitution.	Condition.
Wet	217.6	163.9	26.87	Light.
Do	166.0	166.0	0.0	Dark.
Dry	197.4	197.4	0.0	Light.
Do	166.7	166.7	0.0	Dark.

These figures show conclusively that with the bromin solution moisture and light affect most remarkably the reaction between the oil and the bromin. The fact that a little water added to the solution did not affect the results can be explained on the ground that no mixture of the water with the carbon tetrachlorid solution of the oil had taken place. When the water was mixed with the solution and the flask was in the light a substitution of 26.87 was obtained, but when the flask was set in the dark there was no substitution. The addition figures in both cases are practically the same, but do not agree with the addition figures obtained on the dry oil in the light.

This brings us to the conclusion that the bromin solution does not substitute in the light in the absence of moisture, or at least only very slightly; that it does not substitute in the dark even in presence of moisture, and also does not add to the same extent as it does in the light; that the bromin solution in the light and in the presence of moisture and oil forms hydrobromic acid, the amount formed being dependent almost entirely on the time of contact between the oil and the bromin. The blanks of the solutions showed no formation of acid at all. As the solutions of bromin previously used had always given considerable substitution, fresh solutions of bromin, iodin chlorid, and iodin bromid in carbon tetrachlorid were placed in glass-stoppered flasks and used from day to day to see what effect the age of the solution had on the amount of substitution. The results are shown in Table XVIII.

TABLE XVIII.—*Effect of age of solution on iodin numbers and amount of substitution.*

Age of solution.	Bromin.			Iodin chlorid in carbon tetrachlorid.			Iodin bromid in carbon tetrachlorid.		
	Total.	Addition.	Substitution.	Total.	Addition.	Substitution.	Total.	Addition.	Substitution.
	IN LIGHT ONE HOUR.								
Days.									
Fresh.	197.0	197.0	0.0	198.4	194.2	2.20	189.7	189.7	0.0
2	.....	.....	.....	199.2	194.4	2.40	190.0	190.0	0.0
4	195.7	191.2	2.32	197.9	193.2	2.33	190.3	190.3	0.0
6	200.6	189.9	5.41	200.4	198.7	.86	192.3	192.3	0.0
9	196.2	194.5	.86	.....	.....	.....	.....	.....	.....
10	.....	.....	.....	200.6	193.8	3.44	190.0	188.1	.86
13	219.1	162.9	28.16	.....	.....	.....	.....	.....	.....
	IN DARK ONE HOUR.								
13	166.7	166.7	0.0	.....	.....	.....	.....	.....	.....
10	.....	.....	.....	200.4	194.1	3.18	191.1	189.1	.94

Table XVIII shows how much variation there is in the results by the bromin solution when the same solution has been used. The only varying factors are the amount of light due to weather conditions and the amount of moisture which may be absorbed by the carbon tetrachlorid solution of bromin. The absorption of moisture seems to be the only explanation of the great change which takes place on standing. The titer of the bromin solution remains practically constant, showing only a very slight change. That the amount of light may affect the results by the bromin solution to great extent was shown by the following experiments:

The bromin solution in contact with the oil was exposed to direct sunlight for two minutes and a duplicate was kept in the dark two minutes. The total absorption in the first case was 204.7, addition 166.1, substitution 19.30; in the second case the total was 179.7, addition 165.2, substitution 7.29. On the Wijs and Hanus solutions age has very little effect, and it makes no difference whether the reaction takes place in the dark or light as to the amount of substitution or addition when only thirty minutes are allowed for the reaction; if five hours are allowed, as shown in Table XI, light greatly increases substitution.

These results would indicate that in the light with a dry solution bromin will give the same addition numbers as the Wijs and Hanus solutions, but in the dark it does not give addition values which have any relation to those given in the light. The presence of moisture and light gives results of no value at all. If the determinations are made with dry reagents in the dark comparative results can be obtained, but these results can not be compared with figures given by any other methods. From this work it is concluded that the bromin solution is exceedingly unreliable, being affected by conditions hard to control, that the solutions of iodin chlorid and iodin bromid in carbon tetrachlorid are much less affected by these conditions and give much more satisfactory results which are comparable with those obtained by the same reagents in acetic acid, but that these solutions are affected to a greater or less degree by the presence of moisture and for ordinary work would not be satisfactory substitutes for the Hübl method. The following results on moist solutions of oil show that the iodin chlorid and iodin bromid solutions are not affected by the presence of light or moisture in thirty minutes.

TABLE XIX.—*Effect of light and moisture on iodin numbers with iodin chlorid and iodin bromid solutions.*

Solution.	Total.	Addition.	Substitution.	Conditions.
ICl in CCl_4	200.9	192.4	4.25	In light 30 minutes.
Do	200.9	192.4	4.25	In dark 30 minutes.
IBr in CCl_4	191.2	187.7	1.75	In light 30 minutes.
Do	191.2	187.7	1.75	In dark 30 minutes.

The chief use of any solution of this kind is in the detection of such substances as rosin oil and similar products, as the reaction between the oil and halogen is chiefly by substitution, and a solution which gives varied results would be unsatisfactory. The following results on rosin oil show that the Wijs reagent in carbon tetrachlorid is as active as the bromin, and on account of its other advantages would be a much more satisfactory solution to be used for this determination.

TABLE XX.—*Iodin numbers of rosin oil in 30 minutes.*

Solution.	Total.	Addition.	Substitution.
ICl in CCl_4	257.0	5.46	125.7
IBr in CCl_4	190.8	24.50	83.8
Br	257.0	5.46	125.7

The iodin bromid reacts very slowly and gives quite different results from the other solution, while iodin chlorid and bromin give the same results, so that the iodin chlorid would be the most satisfactory solution for this work. But it does not appear that any of these solutions can be satisfactorily substituted for the Wijs, Hanus, or Hübl in the ordinary work, as they give results not comparing well with present data, require many special precautions, and vary in their reaction with age.

Carbon tetrachlorid, which had been recovered by distilling and drying with calcium chlorid and then redistilling, acted much the same as carbon tetrachlorid, which had been shaken up with water, giving large substitution values. We were not able to prepare a recovered product so that it would give the results obtained with Kahlbaum's C. P. dry carbon tetrachlorid.

To illustrate the widely different results that may be obtained by use of carbon tetrachlorid as a solvent in the Wijs and Hanus solutions under the ordinary conditions, several results have been taken from the large number of determinations made in the course of this work. In Table XXI are the results on an olive oil with a Hübl number of 84.6.

TABLE XXI.—*Iodin numbers on an olive oil with iodin chlorid in carbon tetrachlorid.*

Time.	Total.	Addition.	Substitution.
Minutes.			
5	86.1	85.2	0.45
10	87.3	84.8	2.50
15	87.7	85.2	2.50
30	89.1	85.4	3.70
Hours.			
1	90.8	85.8	5.00
2	94.5	85.0	9.50

The solution used had stood for a few weeks. A fresh solution made up with the same carbon tetrachlorid showed no substitution with linseed oil until it had stood for 1 hour and then very much less than the amount shown in the table with the olive oil. The addition figures, however, remained the same and agreed closely with the Hübl number. The iodin bromid solution in carbon tetrachlorid showed no substitution with olive oils and the amount of substitution with the iodin chlorid solution was very uniform for all the oils. The iodin bromid in carbon tetrachlorid gave higher results than when acetic acid was the solvent, although there was no substitution, while the addition figures with iodin chlorid in carbon tetrachlorid were lower than the figures obtained with acetic acid.

In Table XXII are collected the results obtained on a number of olive oils.

TABLE XXII.—*Iodin numbers of olive oils by different methods.*

Laboratory. No.	Hübl No.	Wijs No.	Hanus No.	Iodin chlorid in carbon tetrachlorid.			Iodin bromid in carbon tetrachlorid.
				Total.	Addition.	Substitution.	
1	83.7	85.0	83.8	88.1	83.4	2.36	84.8
3	79.9	81.3	79.1	84.8	79.5	2.67	80.8
4	86.1	86.6	85.6	90.3	86.1	2.06	86.9
5	83.2	84.4	83.0	88.0	83.0	2.50	84.3
6	84.6	85.4	84.6	90.2	85.0	2.60	85.7
7	90.6	91.5	89.8	94.5	90.2	2.17	.....
10	86.1	87.8	86.7	90.9	85.3	2.82	.....

After reviewing all the work done the associate referee offers the following conclusions:

(1) That much better results are obtained by the Wijs and Hanus solutions than by the Hübl.

(2) That the Hanus gives results much more closely agreeing with the existing data and is easier to prepare, but an excess of 60 to 70 per cent is necessary to obtain quick action.

(3) That the Wijs solution is more rapid in its action, and an excess of 35 per cent is sufficient, effecting a large saving in reagents and time of titrating, but it gives higher results.

(4) That 30 minutes is sufficient time for the action of either the Hanus or Wijs solution.

(5) That acetic acid is a better solvent for the work than carbon tetrachlorid.

(6) That the bromin solution or iodin chlorid or iodin bromid in carbon tetrachlorid are not satisfactory for ordinary work.

(7) That iodin chlorid in carbon tetrachlorid is the most satisfactory solution if determination of substitution is to be made.

(8) That both the iodin chlorid and iodin bromid being much less volatile than the bromin, there is much less danger of loss in that way with the former reagents.

Recommendations.

The associate referee offers the following recommendations:

That the Hübl method as given on page 50 of Bulletin 46, Official Methods of Analysis, and on page 24 of Bulletin 65, Provisional Methods of Analysis of Foods, be dropped, and that the following method be substituted, to be called the Hanus method for the iodin absorption of oils and fats.

(A) *Preparation of reagents.*

(1) *Iodin solution.*—(*a*) Dissolve 13.2 grams iodin in 1,000 cc glacial acetic 99.5 per cent acid (showing no reduction with bichromate and H_2SO_4); add enough bromin to double the halogen content determined by titration—3 cc of bromin is about the proper amount. The iodin may be dissolved by the aid of heat, but the solution should be cold when bromin is added.

(2) *Decinormal sodium thiosulphate solution.*—Dissolve 24.8 grams of chemically pure sodium thiosulphate, freshly pulverized as finely as possible and dried between filter or blotting paper and dilute with water to 1 liter at the temperature at which the titrations are to be made.

(3) *Starch paste.*—One gram of starch is boiled in 200 cc of distilled water for 10 minutes and cooled to room temperature.

(4) *Solution of potassium iodid.*—One hundred and fifty grams of potassium iodid are dissolved in water and made up to 1 liter.

(5) *Decinormal potassium bichromate.*—Dissolve 4.9066 grams of chemically pure potassium bichromate in distilled water, and make the volume up to 1 liter at the temperature at which the titrations are to be made. The bichromate solution should be checked against pure iron.

(B) *Determination.*

(1) *Standardizing the sodium thiosulphate solution.*—Place 20 cc of the potassium bichromate solution, to which has been added 10 cc of the solution of potassium iodid, in a glass-stoppered flask. Add to this 5 cc of strong hydrochloric acid. Allow the solution of sodium thiosulphate to flow slowly into the flask until the yellow color of the liquid has almost disappeared. Add a few drops of the starch paste, and with constant shaking continue to add the sodium thiosulphate solution until the blue color just disappears.

(2) *Weighing the sample.*—Weigh about one-half gram of fat or 0.250 gram of oil [a] on a small watch crystal or by other suitable means. The fat is first melted, mixed

[a] With drying oils which have a very high absorbent power, 0.100 to 0.200 gram should be taken.

thoroughly, poured onto the crystal, and allowed to cool. Introduce the watch crystal into a wide-mouth 16-ounce bottle with ground-glass stopper.

(3) *Absorption of iodin.*—The fat or oil in the bottle is dissolved in 10 cc of chloroform. After complete solution has taken place, 25 cc of the iodin solution are added. Allow to stand, with occasional shaking, for 30 minutes. The excess of iodin should be at least 60 per cent of the amount added.

(4) *Titration of the unabsorbed iodin.*—Add 10 cc of the potassium iodid solution and shake thoroughly, then add 100 cc of distilled water to the contents of the bottle. Titrate the excess of iodin with the sodium thiosulphate solution, which is added gradually, with constant shaking, until the yellow color of the solution has almost disappeared. Add a few drops of starch paste, and continue the titration until the blue color has entirely disappeared. Toward the end of the reaction stopper the bottle and shake violently, so that any iodin remaining in solution in the chloroform may be taken up by the potassium iodid solution.

(5) *Setting the value of iodin solution by thiosulphate solution.*—At the time of adding the iodin solution to the fat, two bottles of the same size as those used for the determination should be employed for conducting the operation described above, but without the presence of any fat. In every other respect the performance of the blank experiments should be just as described. These blank experiments must be made each time the iodin solution is used. Great care must be taken that the temperature of the solution does not change during the time of the operation, as acetic acid has a very high coefficient of expansion, and a slight change of temperature makes an appreciable difference in the strength of the solution. Example blank determinations: (1) Forty cc iodin solution required 62.05 cc of sodium thiosulphate solution. (2) Forty cc iodin solution required 62.15 cc of sodium thiosulphate solution. Mean, 62.1 cc.

Per cent of iodin absorbed:

Weight of fat taken	grams	1.0479
Quantity of iodin solution used	cubic centimeters	40.0
Thiosulphate equivalent to iodin used	do	62.1
Thiosulphate equivalent to remaining iodin	do	30.2
Thiosulphate equivalent to iodin absorbed	do	31.9
Per cent of iodin absorbed ($31.9\times0.012\times100\div1.0479$)		39.61

The following precautions should be exercised in the use of this solution:

1. Great care must be used to prevent change of temperature of the solution, and where any number of determinations are to be made blanks should be measured out at short intervals. This precaution applies as well to the use of the Hübl solution, as the coefficient of the expansion of alcohol is large.

2. When the potassium iodid is added the solution should be thoroughly mixed before the addition of water.

3. The acetic acid must be full strength and pure in order to obtain a solution which will keep well.

The associate referee also recommends that the factor given on page 49 of Bulletin No. 46, Official Methods, for correction for temperature with the refractometer, be changed from 0.000176 to 0.000365 to conform to the correction in Bulletin 65, Provisional Methods, page 22; that under the method on page 32 of Provisional Methods, section 16, in the Halphen test for cotton-seed oil, the brine bath shall boil at from 112° C. to 115° C., and that in making the test for cotton-seed oil the boiling shall be contined for from 1 to 2 hours, the reason for this being that heated cotton-seed oil gives the Halphen test only after long heating, heating from 15 to 30 minutes giving practically no reaction.

For Renard's test[a] for peanut oil, as modified by Tolman, page 33, paragraph 18, Bulletin No. 65, Provisional Methods, the following modified method is recommended:

Weigh 20 grams of oil into an Erlenmeyer flask. Saponify with alcoholic potash, neutralize exactly with dilute acetic acid, using phenolphthalein as indicator, and wash into a 500-cc flask containing a boiling mixture of 100 cc of water and 120 cc of a 20 per cent lead acetate solution. Boil for a minute, and then cool the precipi-

[a] Renard, Comp. rend., 1871, **73:** 1330. Benedikt and Lewkowitsch, Oils, Fats, and Waxes, p. 365.

tated soap by immersing the flask in water, occasionally giving it a whirling motion to cause the soap to stick to the sides of the flask. After the flask has cooled, the water and excess of lead can be poured off and the soap washed with cold water and with 90 per cent (by volume) alcohol. Now add 200 cc of ether, cork the flask, and allow to stand for some time until the soap is disintegrated, then heat on the water bath, using a reflux condenser, and boil for about 5 minutes.[a] In the oils most of the soap will be dissolved, while in lards, which contain is so much stearin, part will be left undissolved. Cool the ether solution of soap down to from 15° to 17° C., and let stand until all the insoluble soaps have crystallized out—about twelve hours are required.

Filter and thoroughly wash the precipitate with ether. Save the filtrate for the determination of the iodin number of the liquid fatty acids by the Muter method. The soaps on the filter are washed back into the flask by means of a stream of hot water acidified with hydrochloric acid. Add an excess of dilute hydrochloric acid, partially fill the flask with hot water, and heat until fatty acids form a clear, oily layer. Fill the flask with hot water, allow the fatty acids to harden and separate from the precipitated lead chlorid; wash, drain, repeat washing with hot water, and dissolve the fatty acids in 100 cc of boiling 90 per cent (by volume) alcohol. Cool down to 15° C., shaking thoroughly to aid crystallization. From 5 to 10 per cent of peanut oil can be detected by this method, as it effects a complete separation of the soluble acids from the insoluble, which interfere with the crystallization of the arachidic acid. Filter, wash the precipitate twice with 10 cc of 90 per cent (by volume) alcohol, and then with alcohol of 70 per cent (by volume). Dissolve off the filter with boiling absolute alcohol, evaporate to dryness in a weighed dish, dry and weigh. Add to this weight 0.0025 gram for each 10 cc of 90 per cent alcohol used in the crystallization and washing if done at 15° C.; if done at 20°, 0.0045 gram for each 10 cc. The melting point of arachidic acid obtained in this way is between 71° and 72° C. Twenty times the weight of arachidic acid will give the approximate amount of peanut oil present. No examination for adulterants in olive oil is complete without making the test for peanut oil.

The associate referee wishes to acknowledge the assistance received from A. F. Seeker, of the laboratory of the Bureau of Chemistry, who practically repeated all of the work in checking up the results obtained, although in the bromin work it was found almost impossible to duplicate any set of results.

In continuation of the report on fats and oils the following paper was submitted by the associate referee:

REPORT OF COOPERATIVE WORK ON THE DALICAN TITER TEST.

By L. M. Tolman, *Associate Referee on fats and oils.*

Work on this test was begun at a very late date, and this report can be considered only as as a preliminary one presented with the idea that by the next meeting of the association some definite and satisfactory method may be offered.

The various modifications of the test in use do not give a satisfactory agreement, and several of the largest concerns in this country using it expressed a desire that an effort should be made by the Association of Official Agricultural Chemists to cooperate with them, as we have with the tannin industry, and prepare uniform methods of analysis so that results obtained by different analysts shall agree. In tests of this kind, which are to a certain degree empirical, uniformity of methods, even as to details, is absolutely essential.

The first step was to send samples of fats to the various men interested, requesting them to report their results and to give in detail their methods. Results were received from 19 chemists representing the following firms in which the test is in constant use: American Cotton Oil Company, Southern Cotton Oil Company, Armour & Co., Nelson Morris & Co., James Kirk & Co., Colgate & Co., Procter & Gamble, Stillwell

[a] Process used by N. J. Lane in his modification of Muter's method. J. Am. Chem. Soc., 1893, **15**: 110.

& Gladding, W. J. Wilcox Lard and Refining Company, Central Lard Company, and Swift & Co. Results were also received from Prof. A. H. Gill, of the Massachusetts Institute of Technology, and G. E. Dyck, of the National Provisioner Laboratory.

The following directions for reporting results were sent with the samples of fat:

State the detail of the method used, also size and thickness of test tube used, and capacity of bottle.

Give the saponification value of the fatty acids.

Give the temperature of the room at time of test.

If possible have thermometer used checked against a standard, also state kind and dimensions of thermometer used, size of bulb especially.

If possible be present at the meeting of the Association of Official Agricultural Chemists, November 19, 1903, in order that there may be a thorough discussion of the results, and a satisfactory method outlined for next year.

Very complete reports were sent in by practically all of the analysts and a large amount of valuable data was thus obtained. It was found from these reports that there are a number of methods of procedure in use in this country. These can be brought under three heads: (1) Modifications of Dalican's method; (2) modifications of Wolfbauer's method; (3) modifications of Finkener's method. As it has long been known that these different methods give varying results it was not expected that the figures obtained would agree closely.

The following modification of Dalican's test, used by James Boyce, of the American Cotton Oil Company, and David Wesson, of the Southern Cotton Oil Company, was practically the same as that employed by 10 of the chemists:

APPARATUS.

A round iron or metallic dish, 1.5 liters capacity.

A porcelain dish, 2 liters capacity.

Test tube, 22 mm to 25 mm diameter, 15 cm long, the walls of the tube to be 1.5 mm thick.

One bottle, 7.5 cm. diameter, 15 cm. deep, to have wide mouth, with cork of sufficient size to hold test tube. Bottle to be of clear glass, with thick wall.

A delicate certified thermometer, reading in $\frac{1}{10}$ degrees, graduated from 0° to 70° C. Bulb of thermometer to be not more than 6 mm. in diameter and 3.4 cm. long.

An accurate thermometer to hang alongside of the apparatus to record the temperature of the room in which test is made.

OPERATION.

Weigh 50 grams of the fat into a metal dish. Measure 40 cc of caustic soda, 36° Beaumé, and 50 cc of 95 per cent alcohol into the small bottle. Heat the fat over a gentle flame to 115° to 120° C. Shake the alcohol and soda solution well together and add to the fat. Stir vigorously over a gentle fire until soap becomes sufficiently dry to form pieces which will no longer adhere to the spatula by gentle pressure.

Redissolve in 20 cc of alcohol and again dry to a condition in which the soap will not adhere to the spatula by pressure.

Transfer the dry soap to the porcelain dish. Add one or two liters of boiling water and keep the mixture boiling for 40 minutes. Replace, by adding cold water, about the same quantity of water which has been evaporated by the previous boiling. Add to the soap solution 70 cc of 25° Bé. sulphuric acid. Boil again until the fatty acids, which will float on top, are perfectly clear and transparent and show no signs of any particle of soap which has not dissolved. Siphon off the acid water and transfer the fatty acids to a small beaker, melt the acids and allow the water to settle; transfer to a dry beaker, dry in a water bath or on a steam bath for 20 minutes and filter the fatty acids through white filter paper, using a hot-water funnel.

Place the clear fatty acids in the titer tube, filling it two-thirds full, the tube being first warmed in the water bath. Insert the tube in the perforated cork of the large bottle, to serve as an air bath, and hang the delicate thermometer so that the bulb will be exactly in the middle, in all directions, of the mass, and allow the fatty acids to cool until the mercury stops falling and just begins to rise again. Then move the thermometer rapidly around the sides of the tube, three times to the right and three times to the left, without any up or down motion, finally replacing the thermometer in the center of the tube.

Watch the thermometer as the mercury rises and take the highest point reached as the titer test, at the same time recording the temperature of the room in which the test was made.

The results on the three samples of fat obtained by this method are collected in Table I.

TABLE I.—*Results obtained by modifications of Dalican test.*

Analyst.	Titer.			Test tube.		Bulb of thermometer.		Method of stirring.
	Sample No. 1.	Sample No. 2.	Sample No. 3.	Diam.	Length.	Diam.	Length.	
Sent by James Boyce, American Cotton Oil Co., Chicago:	° C.	° C.	° C.	cm.	cm	cm.	cm.	
J. B	49.6	40.15	36.30	2.2	15	0.6	3.5	3 times to right and 3 times to left.
C. C. B	49.9	40.3	36.2	2.2	15	.6	3.5	
C. B. C	49.35	40.4	36.33	2.2	15	.6	3.5	
J. H. S	49.40	40.15	36.43	2.2	15	.6	3.5	
A. D. W	49.5	40.40	36.2	2.2	15	.6	3.5	
Sent by David Wesson, Southern Cotton Oil Co.:								
R. E. H	49.6	40.8	36.2	2.5	15	.....	.....	Do.
D. W	49.6	40.4	35.9	2.5	15	.....	.....	
W. J. B	49.7	40.5	36.3	2.5	15	.....	.....	
L. M. Tolman, U. S. D. A	49.3	40.3	36.0	2.2	15	.5	2.0	Do.
A. F. Seeker, U. S. D. A	49.55	40.3	35.75	2.2	15	.5	2.0	Do.
E. G. Halloway, James Kirk & Co.	49.50	40.0	35.90	1.7	15	.4	3.8	Do.
R. D. Oilar, Wilcox Lard Co.	49.58	40.35	36.30	2.5	15	.6	2.3	Do.
E. K. Nelson, Nelson Morris & Co.	49.85	39.95	35.9	3.0	18	.6	4.5	Do.
Theo. Smith, Central Lard Co.	49.45	40.20	35.65	2.5	21	.....	3.0	Do.
A. H. Gill, Massachusetts Institute Technology.	49.50	39.8	37.6	1.2	15	.35	1.7	
Stillwell & Gladding	49.9	40.7	36.7	.....	.....	.....	.....	6 times to right and 6 times to left.
W. D. Richardson, Swift & Co.	49.7	40.7	36.6	.....	.....	.3	1.5	
Maximum	49.9	40.8	37.6	.....	.....	.....	.....	
Minimum	49.3	39.8	35.65	.....	.....	.....	.....	
Average	49.5	40.2	36.1	.....	.....	.....	.....	
Difference between maximum and minimum.	.6	1.0	1.95	.....	.....	.....	.....	

The results in Table I show a fair degree of uniformity, although the details of carrying out the method varied somewhat. But if the results of those using test tubes 2.2 to 2.5 cm in diameter and stirring the acids three times to the right and three times to the left are compared, a much more satisfactory agreement is shown. Twelve sets of results come under this head and the comparison is as follows:

TABLE II.—*Results obtained with uniform test tubes and stirring.*

Determinations.	Sample No. 1.	Sample No. 2.	Sample No. 3.
	° C.	° C.	° C.
Maximum	49.9	40.8	36.4
Minimum	49.3	40.2	35.6
Difference	.6	.6	.9

There are a number of other factors which influence titer, such as moisture and kind of thermometer, and while the above results are fairly satisfactory much better ones would undoubtedly be obtained if uniform methods of drying the fatty acids were used and uniform thermometers. Some of the thermometers used were very long and the correction for the mercury out of the fat would be considerable. Wolfbauer found that the method of saponification did not influence the titer, providing the alcohol was completely removed in alcoholic saponification, but that moisture had a marked influence and titers 0.6 lower were found in moist than in dry fats. He also found that the size of the test tube affected the results, a 2½-cm tube giving results 0.2 lower than a 3½-cm tube, although tubes wider than 3½ cm did not affect the results. These are points which should be considered in our future work.

The details of the work of the various chemists who used the Dalican method will be given in so far as they differ from the method given above. Mr. Boyce and his associates, Mr. Wesson and his associates, Mr. Oilar, Mr. Seeker, and the associate referee followed this method in detail. E. G. Halloway, of James Kirk & Co., makes a slight change. He begins to stir three times to the right and three times to the left when incipient solidification sets in.

E. K. Nelson, of Nelson Morris & Co., gives the following directions:

Weigh out 50 grams of fat into an 8-inch porcelain dish. Melt on a steam bath and saponify with a mixture of 30 cc of 40° Bé. caustic soda and 50 cc. methyl alcohol. * * * Boil until the fatty acids are perfectly clear, collect them, and dry them for two hours at 100° C. * * * Place the thermometer bulb as nearly as possible in the center of the fatty acids and watch the mercury column until it becomes stationary. * * * Then raise the cork from the test tube and stir the fatty acids by moving the thermometer three times in one direction and three in the opposite direction around the wall of the test tube.

Mr. Nelson also makes the following suggestion from his experience:

While with Swift & Co. in the capacity of chief chemist, I observed a discrepancy between two laboratories which was traced to the fact that one chemist used a quick-acting thermometer, short stem with small bulb, while the other chemist used a long thermometer with a large bulb.

Theo. Smith, of the Central Lard Company, makes the following comment:

Any moisture clinging to the cake can be removed with a piece of filter paper, but on no account should the acids be dried between filter papers, as is done by some analysts who obtain results a degree or so above anyone else. Nor should the acids be dried in an air bath, as mixtures containing the acids of cotton-seed oil and other unsaturated acids susceptible to oxidation often give abnormal results if so treated. * * * A few drops of water in the acids make no difference with the titer as they settle to the bottom of the tube and do not interfere with the crystallization.

Prof. A. H. Gill, of the Massachusetts Institute of Technology, followed Dalican's method as given in Lewkowitsch.[a]

Stillwell & Gladding use the French Syndicate method, which is a modification of Dalican's test, and give the following additional modifications:

Decant the clear, fatty acids into a shallow flat-bottom dish, about 4 inches in diameter, and heat for 20 minutes in a water oven at 100° C. This expels the last traces of water, and is not prolonged enough to affect a true titer test.

In stirring with the thermometer at the point where the mercury remains stationary, stir vigorously half a dozen times from right to left, and the same number from left to right. In doing this be careful neither to raise nor lower the thermometer. The object of this is to avoid mixing with the fat acids in the neighborhood of the bulb of the thermometer, the fat acids above the bulb which are hotter, or the fat acids below the bulb which are colder.

The result of our work would lead one to favor the method of constant stirring as the best method for making titer determinations were it not for the fact that in the

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[a] Second ed., p. 133.

oleo-stearine sample the constant stirring method seems to give too low results. This is attributed to the fact that the stearine (crystallizing at so high a temperature) cools off so rapidly that the constant stirring keeps the whole mass below the proper point. When this oleo stearine was tested by the constant-stirring method at a room temperature of 100° F. a reading of 49.90° C. was obtained.

If, however, the stirring is maintained until the mercury stops and just begins to rise, and the thermometer is then allowed to stand quietly in the center of the tube the proper titer point is obtained.

TABLE III.—*Results obtained by Stillwell and Gladding.*[a]

Determination and method.	Oleo stearine.	Edible tallow.	White grease.
Saponification value	204	204	198
	° C.	° C.	° C.
Titer (French Syndicate method)	49.90	40.70	36.70
Titer (constant stirring)[b]	49.70	40.70	37.10
Titer (constant stirring till mercury stops falling and just begins to rise, then stop stirring, and watch mercury as it rises)	49.90	40.90	37.10

[a] Temperature of the room about 70° F.
[b] Using same tube as in French Syndicate method and stirring with horizontal circular motion, without any up and down motion.

The third method appears to be the best one for determining the titer.

Wilson H. Low, of the Cudahy Packing Company, waits until the crystals in the tube begin to surround the bulb of the thermometer, then stirs. He makes the following comment:

We buy fat of other parties who make the test in a larger tube and with continual stirring, and their method makes the titer about a degree higher than ours. As the test is only an empirical one it doesn't make much difference how it is made, but it is desirable for trade purposes that there should be some standard, so that it can be known definitely what is meant when a fat of a stated titer is offered.

Mr. David Wesson reported as follows regarding several different methods of saponification and stirring:

Experiments were planned to determine, first, the effect of two different methods of saponification; second, the effect of reading in different size tubes and different size air baths; third, results obtained by different methods of stirring. These three points seem to be the cause of a great many discrepancies between different observers. Owing to continued warm weather most all the observations were made at room temperatures running from 23° to 28° C. The following methods were employed for saponification:

Method No. 1 for saponification.

Fifty grams fat, 40 cc caustic soda, specific gravity 1.318, 50 cc alcohol 95 per cent, heated together in a 10-inch porcelain evaporating dish on a steam bath. The fat did not reach a temperature above 94° C. The alcohol was evaporated off and the soap run to dryness as shown by breaking up the lumps which no longer stuck to the glass rod.

Method No. 2—Dalican's method.

First method of taking setting point.—Titer as in Dalican's method and use a large tube 25 mm in diameter and an air bath of 1,200 cc capacity.

TABLE IV.—*Results obtained by three analysts with method No. 1.*

No. of sample.	R. E. N.		D. W.		W. H. B.	
	Titer.	Room temperature.	Titer.	Room temperature.	Titer.	Room temperature.
	° C.	° C.	° C.	° C.	° C.	° C.
1	49.9	29	49.9	29	49.8	26
a1	49.6	26	49.6	27	49.7	26
2	40.3	24	40.4	28	40.2	26
a2	40.8	27	40.4	26	40.5	26
3	35.8	23	36.2	25	36.3	26
a3	36.2	28	35.9	25	36.3	26

a Saponified by Dalican's method.

Second method of taking setting point.—Same tubes and bath were used as in method No. 1. The thermometer was stirred slowly, about 60 turns per minute, and continuously until the mercury ceased dropping. The bulb of the thermometer was then left in the center of the mass, and the highest point reached by the mercury was noted.

TABLE V.—*Results obtained by two analysts with method No. 2.*

No. of sample.	D. W.		W. H. B.	
	Titer.	Room temperature.	Titer.	Room temperature.
	° C.	° C.	° C.	° C.
1	50.2	27	50.3	29
a1	50.2	27	50.2	28
2	41.0	27	41.2	29
a2	41.2	27.5	41.4	29
3	37.3	25	37.5	29
a3	37.2	25	36.1	28

a Saponified by Dalican's method.

Third method of taking setting point.—The readings were taken in the same manner as in methods Nos. 1 and 2, except that the air bath had a capacity of 500 cc, and the titer was taken in ordinary test tubes 18 mm in diameter. The results given below show the effect of cooling without stirring, except at the end of the operation, and of cooling by constant stirring.

TABLE VI.—*Results obtained by method No. 3 (W. F. B.).*

No. of sample.	Titer. (Cooled without stirring, except at end of operation.)	Room temperature.	Titer. (Cooled by constant stirring.)	Room temperature.
	° C.	° C.	° C.	° C.
1	49.7	26.6	50.2	26.8
a1	49.7	27.5	50.0	27.4
2	40.2	27.0	41.0	27.5
a2	40.4	27.0	41.2	26.8
3	36.2	27.6	37.3	27.5
a3	36.2	27.0	36.3	27.0

a Saponified by Dalican's method.

Examination of the results seems to show that at the temperature at which the work was done the size of test tube used had no effect.

(2) Higher results were obtained in most cases by constant stirring.

(3) It was noted in making the experiments that it was harder to get definite readings on the soft fat sample No. 3 by constant stirring than by allowing the fat to cool down slowly as in method No. 1.

Three chemists used modifications of Wolfbauer's method.

TABLE VII.—*Results obtained by modifications of the Wolfbauer test.*

Analyst.	Titer of samples Nos.—			Size of test tube.		Size of thermometer bulb.	
	1.	2.	3.	Diameter.	Length.	Diameter.	Length.
	° C.	° C.	° C.	cm.	cm.	cm.	cm.
M. H. Ittner, Colgate & Co	50.47	41.43	37.6	2.6	7.5	0.5	3.0
J. E. Weber, Procter & Gamble	50.00	40.60	.....	4.5	11.0	.4	2.3
David Wesson	50.20	41.20	37.2	2.5	15.0	.....	.....

It will be seen that this method gives higher results than the Dalican method. The details of the operation in Armour & Co.'s laboratory are given by A. G. Manns, as follows:

Seventy-five grams of the fat are placed in an iron dish with 150 cc of caustic soda lye of 13° per cent to 14 per cent strength. The mixture is boiled to dryness with constant stirring so as to avoid scorching. In practice, a 3-pound lard pail is used instead of the iron dish and a heavy stirring rod to break up the lumps. The dry granular soap is then transferred to a porcelain dish of about 1,000 cc capacity, in which 500 cc of water and 250 cc of sulphuric acid of 60 per cent strength have been previously mixed. The whole is boiled until the soap is decomposed, water being added from time to time to maintain the original volume. An enameled cover is kept on the dish while boiling. When the fatty acids are perfectly transparent they are washed several times with boiling hot water, allowed to settle, decanted, filtered through several thicknesses of dry filter paper, and dried for four hours at 105° C. The fatty acids when dried are cooled to about 15° or 20° C. above the expected titer and transferred to a special tube 1 inch in diameter and 4 inches in length. The tube is made of glass about $\frac{3}{4}$ of an inch thick. It is placed in a bottle fitted with a cork which is perforated so as to hold the tube rigidly when in position. The thermometer, graduated to 0.1° C., is suspended so that it can be used as a stirrer, and the mass is stirred slowly until the mercury remains stationary for at least 30 seconds. The thermometer is then allowed to hang quietly with the bulb in the center of the mass and the rise of the mercury is observed. The highest point to which it rises is taken as the titer of the fatty acids.

The fatty acids should always be tested for complete saponification as follows:

About 3 cc of the fatty acids are placed in a test tube and about 15 cc of strong alcohol added, the mixture brought to a boil, and an equal volume of ammonia, specific gravity 0.96, added. A clear solution should result, turbidity indicating presence of unsaponified fat.[a]

It will be noted that the method differs from the Dalican test in two important details. First, the fatty acids are thoroughly dried; second, the method gives a definite time when to stop stirring. The thorough drying of the fatty acids was adopted as given above, as it has been clearly demonstrated that moisture in the fatty acids lowers the titer about 0.4° to 0.5° C. Further, if the fatty acids are dried in hydrogen gas by heating up to 150° C., the titer is the same as if the heating was performed in the open air, or if they were dried in a bath for about four hours at 105° C.[b]

Mr. Ittner uses the following method:

The acids are detached and the dish drained of nearly all its water and the acids melted down in the same dish. After the acids are melted clear, they are poured into a dry bottle and kept on the water bath until the titer can be taken.

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[a] Lewkowitsch, Chem. Anal. of Oils, Fats, and Waxes, 2d ed., p. 100.

[b] Wolfbauer's work, Lewkowitsch, Chem. Anal. of Oils, Fats, and Waxes, 2d ed., p. 134.

The acids in the test tube are cooled down by holding the tube in the hand and stirring with the thermometer. When the temperature falls within a degree or two of the point of incipient crystallization and while they are still clear and fluid, the tube is put into the outer jar and the stirring carefully continued without intermission until very fine crystals begin to form throughout the mass. Then the stirring is stopped and the bulb carefully adjusted to the center of the acids. At this point, there should be no thick masses of crystals, but only a few fine scattered ones. Crystallization started in this way proceeds evenly throughout the mass. The temperature may fall a little, then it usually begins to rise again until a maximum is reached, at which point the reading is constant for several minutes. The mercury then begins to fall, and the maximum temperature reached just previous to this is taken as the titer test. The acids are evenly solidified around the outer parts of the test tube, but the inner portion around the bulb is still quite soft.

One chemist, Mr. G. E. Dyck, of the National Provisioner Laboratory, used a modification of Finkener's method and his results are given below:

TABLE VIII.—*Titer on official samples by Finkener's method.*

Titer of samples Nos.—			Size of test tube.	
1.	2.	3.	Diameter.	Length.
°C.	°C.	°C.	*cm*	*cm*
49.85	40.7	37.35	3.8	10.0

These results are slightly higher than those given by the Dalican method and not so high as those by Wolfbauer's method.

The special features of Mr. Dyck's method are as follows:

When the acids are clear, the underlying water is withdrawn by means of a siphon or the filter pump, fresh, cold water is added and the acids liquefied again until clear. The water is again removed and the solid acids washed twice with cold water and dried with unprinted newspaper, whence they are placed in a beaker and the latter in the water bath. When perfectly liquefied the acids are poured off the remaining water into a dry beaker and once more heated in the bath for 30 minutes. The acids are next filtered through a dry, folded filter paper into a tube 1½ by 4 inches in the drying oven at a temperature of approximately 15° to 20° above the supposed titer. The tube is placed in a paper mailing case and this in a wooden box 3 by 3 by 6 inches, provided with a close-fitting lid, according to Finkener, the empty space being filled with absorbent cotton. The whole is previously placed in the drying oven and warmed to the temperature of the filtering acids.

The perforated lid of the box is closed and at the appearance of the first crystals at the bottom of the tube the acids are stirred by the thermometer to the right and left a few times. When the mercury stops falling the stirring is repeated so that the crystals are uniformly distributed throughout the entire mass, without, however, touching the sides of the tube with the thermometer. The stopping point is noted as well as the high point reached by the mercury, but the latter only is reported.

It is evident from this work that there is need of a uniform method of procedure if agreeing results are to be obtained. The reports show that the two methods in general use are Dalican's and Wolfbauer's, and it was thought by those present at the meeting that these two methods would furnish a satisfactory working basis for the coming year, the idea being that these methods should be tried and the one giving the most satisfactory results be recommended for adoption as the official method.

The PRESIDENT. If there is no discussion, we will take up the subject of fruits and fruit products.

REPORT ON FRUITS AND FRUIT PRODUCTS.

By L. S. Munson, *Associate Referee.*

The directions given in Bulletin No. 65, Provisional Methods of Food Analysis, page 78, for the polarization and determination of cane sugar, differ from the method for these operations to be submitted at this meeting, by the referee on sugar, as the official method. I recommend, therefore, that the methods on page 78, under fruits and fruit products, for polarization and the determination of cane sugar be made to conform to the official sugar methods, that there may not be two different methods for making the same determination. For the determination of reducing sugars the Allihn method is given (sec. 14, p. 78), for which should be substituted the regular official method, which is the Meissl method used with the Meissl and Hiller tables, and this method should be used also for the determination of cane sugar by the process of inversion.

On page 78, section 15, is given a method for the determination of dextrin. The only object in determining dextrin in fruit products is to arrive approximately at the amount of glucose in the materials. Since Bulletin 65 was published, two years ago, a method has been suggested by A. E. Leach, and another by Edward Gudeman, for the determination of commercial glucose in mixtures of this nature. Mr. Leach's method is printed as provisional on page 48 of Bulletin No. 65. Mr. Gudeman's method may be found on page 65 of the Nineteenth Annual Proceedings of this association for 1903, Bulletin No. 73. I believe that both of these gentlemen have some remarks to make concerning these methods. I have no further recommendations to make concerning fruits and fruit products.

The President. I suggest that we take up the subject of saccharine products in order to give the gentlemen an opportunity to present the papers or remarks on glucose.

THE DETERMINATION OF COMMERCIAL GLUCOSE IN SOME SACCHARINE PRODUCTS.

By Albert E. Leach.

The determination of commercial glucose in such products as molasses and maple sirup, wherein the amount of invert sugar is comparatively small, is readily accomplished by some such means as the provisional method already given in Bulletin 65, but its determination in such products as honey, jams, and jellies presents some obstacles.

At first sight it would seem to be only necessary to divide the invert polarization of the sample at 87° C. by the assumed factor for the polarization of commercial glucose to obtain the percentage of the latter. As a matter of fact, a normal solution of commercial glucose polarizes somewhat lower after than before inversion, and the reading at 87° is very much lower than that at 20°. The difference between the readings at the high and low temperature may be due in part to expansion, but another disturbing influence enters in to affect the results, namely, the action of the acid used in inversion on the maltose and the dextrin of the glucose at high temperatures.

If a normal solution of commercial glucose be subjected to inversion in the ordinary manner, it will be found impossible to obtain a constant reading at a temperature of 87° C. The polarizing figure drops quite rapidly, due no doubt to the hydrolyzing action of the hydrochloric acid on the maltose and the dextrin. On this account it becomes necessary, when analyzing samples of honey, jams, and jellies

containing commercial glucose, to neutralize the sample as soon as possible after inversion, before the hydrolytic action of the acid has set in to any appreciable extent.

It is found best to prepare separate normal solutions of the sample on one of which the direct polarization is obtained in the usual manner. For the invert readings the second solution is prepared as follows: Weigh out half the normal weight of the sample, 13.024 grams, in a 100 cc graduated flask. Dissolve in about 70 cc of water and add 7 cc of concentrated hydrochloric acid. Then heat to 68° C. and cool in the usual manner for inversion. Add a few drops of phenolphthalein, and enough sodium hydroxid to neutralize. Discharge the pink color with a few drops of dilute hydrochloric acid, cool again, add from 5 to 10 cc of the appropriate clarifier, which in the case of honey would be alumina cream, and make up to the mark. Constant and presumably correct readings may be made with this solution both at room temperature and at 87° C. using the 200 mm tube and multiplying the readings by two.

Following are the results of a series of readings made on five samples of commercial glucose by H. C. Lythgoe, showing the relation between the two invert readings at 22° and 87°, respectively, as well as the ratio of the invert polarization at 87° to the direct polarization. The readings were made on solutions subjected to the above method of inversion and neutralization.

TABLE I.—*Polarizations of 5 samples of commercial glucose.*

Source.	Density.	Polarization.				
		A Direct.	B Invert at 22° C.	C Invert at 87° C.	Ratio of C to B.	Ratio of C to A.
Boston Molasses Co.:	°*Bé.*					
No. 1	42	156.6	153.4	146.6	0.956	0.936
No. 2	42	158.6	154.6	149.0	.964	.940
Illinois Sugar Refining Co	42	169.6	165.4	159.4	.964	.940
New England Confectionery Co	43	167.4	162.8	155.0	.952	.926
Illinois Sugar Refining Co	45	174.0	171.0	161.2	.943	.927
Average					.956	.933

Obviously, then, if we are to express results in terms of commercial glucose, polarizing at an assumed figure (for example, 175 as in the provisional method), it will be necessary in the case of jellies and jams to divide the high reading at 87° C. after inversion by 93 per cent of the assumed factor, or if 175 is the factor by 163. It is obvious also that the calculation of invert sugar in products which contain also considerable commercial glucose is a difficult one, by reason of the influence of the commercial glucose on the polarization. We are at present working on this problem and hope before long to be able to work out a reliable formula for the calculation of invert sugar in the presence of commercial glucose.

It has been thought best, however, at this time to call attention to these peculiarities of commercial glucose, in view of the fact that it is understood that the provisional method, while well adapted for molasses and sirups, is being quite largely used for jams and jellies as well. No doubt a rough approximation can be made by the use of the unmodified provisional method for all these products, but where verification of manufacturers' formulas is involved, or where more exact methods are needed, the above precautions should be borne in mind.

The PRESIDENT. We are indeed fortunate in having with us this morning one who is doing so much for the agricultural interests of the country and who has always taken a deep interest in the work of our

association. It affords me great pleasure to introduce the honorable Secretary of Agriculture, Mr. James Wilson, to the association. (Applause.)

ADDRESS OF SECRETARY WILSON.

Mr. President and Gentlemen of the Association: The Secretary of Agriculture can do very little; one man can do very little anywhere, unless he is well sustained in his efforts along the line of his work. I want to say to you that it has been a matter of great satisfaction to myself and the Department of Agriculture to have such steady, unflagging support as we have had from this association. We regard you as among our most intelligent friends. The work that you have been doing has been of great value to the country and is beginning to bear fruit.

We are having a good measure of success in executing the law with regard to the importation of foreign foods that are not good enough for the people who live in the countries where they originate. The conclusion has been reached that they are not good enough for us, and new light is being brought to the subject daily in that the merchants who import those goods are learning that they need the protection of the American chemists. They do not know when goods are adulterated; at least, they have not known, but they are discovering it now. The law is being successfully put in force and the merchants of the United States give it a hearty welcome, and it will work a complete cure in a few years.

I am well satisfied that the American merchants will not endeavor to surreptitiously bring into the United States things that should not be brought in—that are contrary to law. I believe that a very large proportion of the American people are honest people. But we have to admit that once in a while we have a rascal among us and he has to be looked after a little—but I believe he is the extreme exception.

We must take another step. The States are not able to protect themselves and enforce their food laws beyond their own borders. I really think we have come to the time when the Federal Government should give us interstate pure food laws, so that our people may be protected from an occasional rascal we may have at home. I hardly think that we can claim that nobody in the United States is disposed to cheat his neighbor; we are not that near the millennium yet. But this association is aiding us to reach conclusions which I think will help us to discipline the people at home who require discipline. I believe the time has come, and the steady pressure exerted by the gentlemen of this association has brought it about to a large extent, when we should look after the movement of these matters in their relation to interstate commerce.

I have only a moment and you are busy people. I only came to take my hat off to you and give you a hearty welcome and say good day. (Applause.)

The discussion on glucose methods was then resumed.

Mr. Leach. Mr. Gudeman and I have had a friendly controversy with reference to glucose methods. His method is based on the hydrolysis of sugars and mine on the simple polarization. When dealing with such a complex substance as commercial glucose it is impossible to get an exact method for its determination in saccharine products, as its moisture content, polarization, and density all vary. But at the same time we are confronted with the fact that it is necessary sometimes in court work and in verifying formulas on packages for the chemist to adopt some method and express the results in percentage, no matter how unsatisfactory they may be. Therefore, I think the method chosen should be accepted subject to its limitations and the results

given in the terms of some arbitrary factor. The factor assumed in my formula, i. e., the highest polarization of a given grade of commercial glucose used by dealers that, within our experience, has ever been found, gives the manufacturer the benefit of the doubt in every case. If we find 75 per cent of glucose the chances are that the manufacturer has used about 80 per cent. That is, he has used a lower polarizing glucose than we credit to him. At best this is unsatisfactory, but from the commercial standpoint the ease with which the amount of glucose is calculated should be considered. The sample must be polarized in any case, and having done this, the per cent of glucose may be calculated by the formula and the facts obtained quite as easily as by Mr. Gudeman's method based on the hydrolysis of sugars.

Mr. GUDEMAN. The method which I suggested was not offered as a provisional method, but simply as a method giving absolute results. If you compare it with Mr. Leach's method you will see that his method has all the advantages of rapidity, simplicity, and cheapness. But when it comes to absolute accuracy, the method I suggested of determining the component parts of glucose gives closer results. At the same time a very slight modification of Mr. Leach's method will lead to these results, and that modification consists in accepting a constant for the polarization of glucose, and also defining the percentage or accepting a constant for the percentage of water in glucose, and using these two factors irrespective of the article under examination. The whole gist of my original paper is found in a few lines, on page 68, of the proceedings of this association for last year, where I stated that the use of different factors of rotation, subject to the samples under examination, will lead to greater errors than the use of a constant (175), even if the same is not correct. The main criticism I have to make of Mr. Leach's method is that when he examines certain products the constant 175 is used, for other products 150, and in another case 185. Now, if this association will simply define glucose in terms of percentage of water, irrespective of whether it is on a basis of dry substance or the maximum limit of water, 22.5 per cent, and set a standard of polarization, the results obtained by different chemists will agree. As it is now they do not agree. Some chemists are using Mr. Leach's method, and others, among them official chemists, are using the factor 175 for all products that contain glucose. If you recalculate the percentages published you will see that the factor is always between 175 and 176. Mr. Leach is under the impression that various grades of glucose are manufactured for various purposes. That is not so. There is only one kind of glucose manufactured in the United States to-day, but there are different degrees of moisture and the polarization is affected thereby.

I would suggest that this association adopt a constant. I did not come prepared with a paper, but wanted to bring out the fact, and it has been brought out, that the methods of determining glucose are now under consideration by the various chemists, and I believe it will not be long before a method is adopted that will give at least comparative results by different analysts.

Mr. WILEY. What Mr. Gudeman has said in regard to this matter is without question, assuming that his statement, that there is only one grade of glucose made in the United States which varies only in the percentage of water present, is a correct one. I do not mean to accuse Mr. Gudeman of making incorrect statements, but that would be a very difficult thing to secure. It is true that glucose is practically made under the direction of a single firm, the Corn Products Company, which undoubtedly always uses the same processes, and hence his statement comes nearer being true now than ever before in the history of glucose making.

When, in 1880, glucose first became a prominent element in commerce, I was called upon to make some examinations of this substance, and discovered that there was a constant relation between the reducing power of glucose on copper salts and its polarizing power. If all glucoses are reduced to the same standard of water or calculated to the dry substance, you will find an exact relation between the copper-reducing power and the polarization. So exact is this relation that you can polarize a glucose and calculate the amount of copper it will reduce, or vice versa. But even under a single direction there must be differences in the amount of conversion in the starch. It is not technically possible to stop every conversion at exactly the same point. That is the only flaw in Mr. Gudeman's reasoning. If the conversion or hydrolysis were always carried to the same point, then a constant factor could be adopted which would be perfectly satisfactory. But otherwise, a constant factor can not be used. There is no doubt but that if all chemists would take the same factor their results would agree. There is a difference between having results that agree and results that are correct. You can make the the most incorrect results agree, and you can make the most correct results disagree, if you change the subject on which you are working. So I would like to ask Mr. Gudeman and Mr. Leach, is it true that all glucose made now is subjected to the same degree of hydrolysis?

Mr. GUDEMAN. Mr. Wiley is correct in his statement. When I said that there was only one kind of glucose made I did not refer to the chemical composition of the glucose but was answering Mr. Leach's statement that there is one grade of glucose made for the jam manufacturer, another for the jelly manufacturer, and yet others for the brewer and the confectioner. The manufacturer makes only one grade of glucose and that is used by all the different manufacturers.

But this one grade of glucose will vary. I have shown in my article that the variation in the composition of glucose is very large, ranging, as regards the reducing power, from about forty to sixty. Mr. Wiley is correct; it is absolutely impossible for the manufacturer to duplicate the article. For practical purposes they are the same. But theoretically and chemically they are not the same.

The point I want to make is that the manufacturer only makes one grade of glucose to go on the market for the different submanufacturers. This glucose varies right along, and while theoretically the reducing power and the polarization have a certain ratio, and one can be calculated from the other, as a matter of fact it has never been found possible to control the manufacture of glucose by means of the polariscope, though it can be controlled by the iodin test.

Mr. Leach. I think the statement made by Mr. Gudeman that only one grade of glucose is manufactured might create a wrong impression. I do not think he means to say exactly that, because he knows that 45°, 43° and 42° glucose is sold to the trade—that is, there are three distinct grades. Of course, in one sense what Mr. Gudeman says is true, there is only one substance called glucose, but it is supplied to the trade in different grades. I happen to know that the New England Confectionery Association, which has absorbed all the large confectionery establishments in New England, uses only the 43° and 45° Bé. glucose. I also know that molasses mixers use only the 42° grade. Recently I visited the warehouse of the largest manufacturers of compound molasses in New England where, among 200 barrels of glucose all marked 42° Bé., I selected samples at random for analysis. The 45° Bé. glucose, although a liquid, is of such a consistency that you could turn a bottle of it upside down and it would not flow out for half an hour. It is so stiff that it can not be used in molasses, while the 42° Bé. glucose has just the right consistency for that purpose. That is, 45° Bé. glucose to be mixed with molasses must be watered down to the consistency of that product and then it virtually becomes 42° Bé. glucose. In the first place the 45° grade has a polarization much higher than 175, but when watered down the polarization decreases. So, I can not understand how there can be said to be only one grade of glucose when these three grades are made, all varying within certain limits, and are selected for use in the trade with special reference to their consistency.

Mr. Gudeman. On page 66 of your report of last year the subject is covered completely. When I say there is one grade of glucose, that is absolutely correct. You can figure on one grade and you can add as much water as you like and it does not change the glucose. In my original article I give there the extremes for the manufacture of glucose, ranging from 40 parts reducing substance to 60 parts of non-

reducing substance against 55 parts of reducing substance and 45 parts of nonreducing substance. Then I go further and say—

> Glucose is a thick sirup, 41° to 45° Bé, containing 13 to 22.5 per cent of water. * * * If the conversion of starch with acid is carried to a point where a dilute iodin solution will just give a distinct color reaction, we have glucose. * * * The ratio of the reducing substance to nonreducing substance depends on the accuracy in stopping the conversion, for neutralization, at the exact point decided upon. This ratio determines whether the product is glucose or grape sugar, and no sharp dividing line exists. The rotating powers of glucose and grape sugar depend absolutely on this ratio. Actually no two batches of commercial glucose or grape sugar are identical; for all practical purposes they are alike, as a few points either way from the standard decided upon will make no difference in the appearance, taste, or working qualities of these products.

Now, that is the only difference. When you examine a product that contains the glucose it is absolutely impossible to tell what grade of glucose has been used, allowing that each percentage of water means a different grade. If the results are figured on the dry substance, as far as the commercial article is concerned, but one grade of glucose exists. The commercial article itself contains varying percentages of water. When a product is examined it is impossible to determine whether a grade of 41°, 43°, or 45° glucose has been used.

Mr. Leach. I only want to say one more thing and that is that each manufacturer uses the grade that is particularly adapted for his purpose. He is not going out of his way to change the material purchased by watering or boiling down. If he wants a thick glucose he buys the 45°, if he wants a thin glucose he buys 42°.

Mr. Wiley. There is just a little flaw in the cogent reasoning of Mr. Gudeman, and to illustrate it I will say that on the Board of Trade of Chicago, in which city Mr. Gudeman lives, there are three grades of wheat recognized in the market. I have no doubt that in each one of those three grades there are plenty of grains that belong to the other two grades, and yet the three grades are absolutely recognized, and I think we could not say that there is only one grade of wheat recognized on the Chicago market. They have distinct qualities, and each grade can be recognized by experts. So glucose has distinct grades, which can be recognized by the chemist, though the only variation be in the amount of water.

Mr. Gudeman. The German Government recognizes only one grade of glucose as having a certain specific gravity and consequently a certain percentage of water, and I only recognize one grade of glucose free from water, and base my remarks on that point. What Mr. Wiley says is true, but I would like to ask him how he can determine in a glucose mixture whether 41°, 42°, or 45° glucose has been used, and how he can differentiate them when mixed with molasses or any other product?

Mr. WILEY. It has been shown that the difficulty of incorporating a given grade of glucose in certain products is so great that it is not attempted, and therefore if we should find glucose in a certain mixture we would infer that it was not, let us say, 45° glucose.

Mr. LEACH. I would like to make a recommendation that the method as it now stands be changed, to the effect that the results shall be expressed in terms of glucose polarizing at 175°, thus doing away with any uncertainty. I will frame such a recommendation and submit it to the committee on recommendations.

Mr. WILEY. I think that will be a step toward the solution, and it makes no difference whether the result be expressed as hydrous or as anhydrous glucose. It is a good suggestion to regard the solid substance only, and thus remove the difficulty.

Mr. LEACH. Except that when we are called upon to express the quantity of commercial glucose in a mixture, in court, the statement as to the dry substance is not desired.

Mr. WILEY. It must be calculated to the commercial grade of water.

Mr. LEACH. Yes; whereas if one determination is made and expressed as wet substance direct the whole ground is covered at once.

Mr. GUDEMAN. As I am not a member of this association, I could not make a motion, but I think the recommendation made by Mr. Leach covers the whole subject, and it agrees absolutely with my original suggestion that a constant factor be used rather than a variable one. I wish to express my thanks to the association for allowing me to take part in this discussion.

Mr. MUNSON. I would like to say a word or two in regard to the factor selected. In Mr. Gudeman's article in last year's proceedings the range in polarization of the dry material given for glucose is from 186° to 198°. The amount of water is stated to vary from 13 to 22.5 per cent. Assuming that the highest polarization of 198° is found with a glucose having the lowest percentage of water, and reducing the reading to the basis of the original glucose having 13 per cent of water, the highest polarization becomes only 172°, which bears out Mr. Leach's more recent work in which he has shown that the polarization of glucose is very much lower than formerly. It seems probable that the factor 175 is higher than that of any sample of glucose, even the 45° Bé., to be found in the trade.

Mr. LEACH. It is true that later experiments seem to indicate that 42° Bé. glucose as now made polarizes lower than formerly. I selected 175 because that is the factor we have used for ten years, using the highest factor so as to give the manufacturer the benefit of the doubt. When asked in court how much glucose is present, we say, "at least" a certain per cent; there may be more. It is for this reason, and to avoid uncertainty, that I would recommend expressing results in terms of a certain factor. If it seems to be desirable to change this factor at any time it can be done.

The PRESIDENT. Committee C, of which Mr. Leach is chairman, has this subject under consideration, and any further material on the subject can be submitted to him.

The report on dairy products is in order.

Mr. CAVANAUGH (referee on dairy products). It has been impossible for me to make a formal report this year. I recommend that the referee for next year take up the same subject, viz, the effect of preservatives on the albumin of milk with special reference to their quantitative determination.

Mr. PATRICK. I wish to submit a note on the Waterhouse test for the detection of renovated butter, as modified at the Department of Agriculture.

NOTE ON THE WATERHOUSE TEST, MODIFIED, FOR THE DETECTION OF RENOVATED BUTTER.

By G. E. PATRICK.

Two years ago I reported to the association the results of my work with this test as applied to the detection of renovated butter, and in view of certain experiences with the test since that time it seems advisable to make a further report.

In order to avoid confusion, it may be well to review the following facts: The Waterhouse test was originally proposed for the detection of oleomargarin,[a] and consisted in melting the butter or oleomargarin in hot milk (preferably skim milk) and stirring the mixture rapidly while it is cooled by placing the vessel in cold water or otherwise. The writer soon found that renovated butter would behave the same as oleomargarin upon the application of this test—that is, it would gather in a lump or single mass during the stirring unless the cooling was effected very rapidly, as by ice water. In order to make the test one for oleomargarin as distinguished from both renovated and genuine butter, and at the same time to make it useful to butter consumers who need such a test more than chemists do, household utensils were used and a practical way determined of obtaining the temperature, with those utensils, at which oleomargarin gathers in a lump, and butter, either genuine or renovated, granulates.[b] So much for the test as applied to the detection of oleomargarin.

Further, it was found that upon cooling much more slowly than is required in testing for oleomargarin, for example, with water at 12° C., instead of with ice water, renovated butter will gather in a lump or single mass, while, as a rule, genuine butter will granulate. The exceptions to the rule were thought to be butters that had been more or less melted, though possibly others were included, since a few butters not visibly damaged were among the exceptions. These results were obtained when testing with milk. Mr. Wiley suggested trying water instead of milk, and this worked so well that the former medium has been used in the greater part of our work ever since. The details of the test as conducted in this laboratory are as follows:

One hundred cubic centimeters of distilled water in a small tin cup, $2\frac{3}{4}$ inches in diameter, are heated to just below 50° C., a slightly rounded teaspoonful of the butter is added, and gently stirred until melted; the temperature is then raised to exactly 50°, the cup is immediately placed in a pan 9 to $9\frac{1}{2}$ inches in diameter at the base, containing 1,000 cc of water at 12° C. The contents of the cup are then stirred rapidly with a wooden rod (more slender than a match) for 10 minutes with a circular

[a] J. Amer. Chem. Soc., 1901, p. 200.

[b] U. S. Dept. Agr., Farmers' Bul. No. 131.

and crosswise motion in turn, in such manner as to keep the fat well agitated. The water in the pan is thoroughly mixed once every minute, the cup being used as a stirrer. At the end of 10 minutes' stirring the test is finished.

In my report of two years ago it was stated, as the result of experience up to that time, that a butter which granulates in this test is above suspicion of having been renovated, and is surely genuine. Two years more of experience have shown that this view must be modified, for four samples of canned oleomargarin from Europe have been encountered that granulated like genuine butter in this test. Furthermore one sample of canned butter (No. 291 D. D.) also from Europe, which, while it was not a renovated butter judging from the microscopic examination, acted like one in the boiling test, but nevertheless granulated like a genuine butter in the modified Waterhouse test. The fat of this butter, examined after some months exposure, however, gave a Reichert Meissl number of 22.4 and a saponification number of 229.7, figures which certainly fail to prove adulteration. As to the four oleomargarins, it is probable that their behavior was caused by the kind of fats entering into their composition, since other European oleos opened at the same time behaved exactly as do American oleos—that is, they "gathered" in this test.

The failure of the test to detect oleomargarin is of little consequence to the chemist, since he has so many ready means of detecting an oleo. Failure to detect a renovated butter would be much more serious, but in the case cited it is an open question whether or not the test failed at all. The instances given above are the only ones yet encountered in which a butter that granulated in the test was not considered upon other grounds as being genuine, i. e., not renovated—and at least not grossly adulterated. What would be the effect of moderate adulteration in the churn, without melting, is an unanswered question.

Turning now to a consideration of the exceptions, it is found that it is as yet impossible to name with certainty all the causes for the behavior of the few genuine butters that gather in this test. From the first the writer has thought that partial melting of the butter by careless treatment while in transit or storage is one of the causes, but this is merely an assumption, as it has never been proved. Another cause recently discovered by the writer is of a similar nature and was observed in the following way: During the past year frequent analyses were made of butter from a dairy conducted on creamery methods, and it was found that this butter, although fresh and perfect in appearance, invariably gathered in a lump when subjected to the Waterhouse test, modified. This butter had two evident peculiarities; it was always without salt and was of a very mild flavor, so mild in fact that it was at once pronounced to be a sweet cream butter. That the absence of salt was not the cause of the abnormal behavior of the butter in this test was proved by repeating the trial after having salted the butter in the laboratory, the result being the same. For a long time the writer forced himself against reason to almost believe that the peculiar behavior of the butter was due to the fact that it was made, as was believed, from unripened cream, but finally the thought suggested itself that the butter might be made from pasteurized cream, and upon inquiry it was learned that about half of the cream used in the making of each lot of butter was pasteurized at 160° F. for 10 minutes, then mixed with the other half which was not pasteurized, and the whole ripened to an acidity of about 0.6 per cent, a fact which quite upset the theory that it was sweet cream butter.

Since these facts were ascertained the writer has tested samples of all the butters to be found in the Washington market which it was claimed were made from pasteurized cream. Only four brands were found, three in prints, the wrappers of which bore the statement that the butter was made from pasteurized cream; the fourth was tub butter, whose claim to pasteurization rested upon a verbal statement said to emanate from the manufacturer. Of these four samples only one gathered in the test; the other three granulated, thus placing themselves above suspicion. The question naturally arises as to whether these three butters were really made as alleged from

pasteurized cream, and again whether, if so made, the temperature of pasteurization was not lower, or the time of its application shorter, than obtained in the manufacture of the samples which gathered in the test. For the answering of these questions there has as yet been neither time nor opportunity. In whatever way they may be answered in the future, or whatever facts may be developed, certainly those already observed indicate strongly that one of the causes which may cause a genuine butter to gather in the modified Waterhouse test is the pasteurizing of the cream from which it is made.

The PRESIDENT. If there are no other papers on this subject we will take up the report on nitrogen.

REPORT ON THE DETERMINATION OF NITROGEN.

By F. W. MORSE, *Referee.*

Eight laboratories cooperated in the work on available organic nitrogen. Some preliminary work on the neutral permanganate method was executed by Mr. L. A. Hill, of the New Hampshire station, and the department of foods and feeding of the Massachusetts station made some comparisons of various modifications of the alkaline method for determining availability.

As a result of these trials, the referee decided to use but two samples of materials, to leave the neutral method as it stood last year, and to try three modifications of the alkaline method. The preliminary work on the neutral method showed that marked variations in the effect of the permanganate solution on the nitrogenous substance were produced by changes in temperature, such as would be caused by a simmering water bath, or one boiling vigorously, and the use of hot solutions at the start instead of cold. This last modification produced such a marked effect on the solubility of the nitrogen, and increased so much the apparent availability, that it was deemed inadvisable to use it, but to emphasize the importance of a large water bath well filled and boiling vigorously.

The work with the alkaline permanganate solution showed the necessity of a fixed amount of distillate and the probable advantage of a larger volume of solution. The methods as sent to the various analysts were as follows:

The neutral permanganate method.—Into a 400 cc Griffin beaker, low form, weigh an amount of sample containing, approximately, 0.075 grams of nitrogen (samples containing material that has been treated with acid should be washed on a 9 cm S. S. No. 595 filter to 200 cc and transferred, filter and all, to beaker); digest this with 125 cc of permanganate of potash solution (16 grams of pure potassium permanganate in 1,000 cc of solution) in a steam or hot-water bath for thirty minutes. Have the beaker let down well into the steam or hot water and keep closed with a cover glass, stirring twice at intervals of ten minutes with a glass rod. At the expiration of the time remove from bath, add 100 cc of cold water, and filter through a heavy 15 cm folded filter. Wash with cold water, small quantities at a time, till total filtrate amounts to 400 cc. Dry and determine nitrogen in residue by Kjeldahl method.

The alkaline permanganate method.—Weigh out an amount of the sample containing, approximately, 0.045 gram of nitrogen, and transfer to a 600 cc distillation flask. After connecting with a condenser to which the receiver containing standard acid has been attached, digest with 100 cc of alkaline permanganate solution (16 grams of potassium permanganate and 150 grams of sodium hydrate in 1,000 cc of solution) for thirty minutes below the boiling point. Then boil slowly until the distillation is completed.

Since the samples contain superphosphate, it will be necessary to wash them as directed for the neutral method, but it is unnecessary for the alkaline method.

In the neutral method the time and temperature are important. The water bath should be well filled and boiling vigorously. If nearly empty, or barely boiling, it will take a longer time to bring the solution to its maximum temperature, and thus

cause a marked variation in results. Filtering may be quickly done with the aid of a Hirsh funnel and filter pump.

Analysts are requested to try the following modification with the alkaline permanganate solution: Use 150 cc of solution, and after digesting 30 minutes distill off 100 cc. This method varies in results with varying amounts of distillate; consequently a uniform amount of distillate should produce more concordant results. Also try the same quantities of solution and distillate, omitting the thirty minutes digestion.

Dried blood and cotton-seed meal were again selected as types of organic materials to be used for testing the methods, since it was the referee's plan to get more concordant results from the different laboratories than were obtained last year. Both materials were sifted through a 0.5 mm sieve and mixed with an equally fine acid phosphate. The mixing was done as thoroughly as possible, but as the blood contained many hard granules it was expected that the different samples would vary somewhat in their content of total nitrogen, and this proved to be the case.

Nine analysts reported results on total nitrogen and eight on the various methods for the available. The results are given in the tables under the designations of the respective materials.

TABLE I.—*Total and available nitrogen—dried blood, sample No. 1.*

Analyst.	Total nitrogen.	Neutral method.		Alkaline method: Available nitrogen.		
		Insoluble nitrogen.	[a] Available nitrogen.	[b] 1.	[c] 2.	[d] 3.
T. C. Trescot, U. S. Department of Agriculture	[e] 4.20	2.06	2.19	2.40	3.01	3.04
	[f] 4.25	2.03		2.42	3.11	3.04
Department of foods and feeding, Hatch Experiment Station, Massachusetts	4.31	.54	3.78	2.66	2.90	.....
		.52		2.70	.....	.....
				2.64	2.92	.....
F. A. Urner, State Agricultural Experiment Station, New York	4.26	.93	3.41	2.64	2.87	2.91
		.94		.....	.....	.....
		.67		2.65	2.97	2.77
A. B. Foster, Maryland Agricultural College	4.05	.68	3.37	3.04	2.84	2.32
				2.96	2.91	2.34
S. H. Sheib, Virginia-Carolina Chemical Co	[g] 4.29	.44	3.84	.....	.....	.....
		.41		2.66	2.59	.....
	[h] 4.28	.43		2.65	.....	.....
		.46		2.82	2.66	.....
C. H. Jones, Agricultural Experiment Station, Vermont	4.35	.65	3.70	2.79	2.99	2.95
F. M. Hollister, Agricultural Experiment Station, Vermont	4.34	.48	3.86	2.80	2.98	2.94
L. A. Hill, Agricultural Experiment Station, New Hampshire	4.09	.50	3.59	2.65	3.26	3.31
		.49		2.59	3.37	3.32
W. H. Scherffius, Agricultural Experiment Station, Kentucky	[i] 4.27	.45	3.87	2.04	.....	.....
		.37		1.95	.....	.....
		.39		2.36	.....	.....
F. B. Carpenter, Virginia-Carolina Chemical Co	4.25	.....	.....	.....	.....	.....

[a] Calculated by subtracting average of insoluble nitrogen from average total nitrogen.
[b] 100 cc solution, digested 30 minutes, distilled.
[c] 150 cc solution, digested 30 minutes, distilled 100 cc.
[d] 150 cc solution, no digestion, distilled 100 cc.
[e] Total by Gunning method; digested 4 hours.
[f] Digested 5 hours.
[g] Digested 1½ hours.
[h] Digested 2 hours.
[i] Digested 5 hours.

TABLE II.—*Total and available nitrogen—cotton-seed meal, sample No. 2.*

Analyst.	Total nitrogen.	Neutral method.		Alkaline method: Available nitrogen.		
		Insoluble nitrogen.	[a]Available nitrogen.	[b]1.	[c]2.	[d]3.
T. C. Trescot, U. S. Department of Agriculture	[e]3.81 [f]3.81	.47 .47	3.34	1.52 1.52	1.89 1.92 1.89	2.01 1.96
Department of foods and feeding, Hatch Experiment Station, Massachusetts	3.83	.25 .24 .24	3.59	1.63 1.62	1.88 1.86	.....
F. A. Urner, State Agricultural Experiment Station, New York	3.85	.48 .59 .50	3.33	1.89 1.82	2.25 2.34	2.19 1.96
A. B. Foster, Maryland Agricultural College	3.89	.48	3.41	1.77 1.86	2.38 2.47	1.37 1.37
S. H. Sheib, Virginia-Carolina Chemical Co	[g]3.80 [h]3.77	.21 .24 .24 .22	3.57	1.67 2.14 1.87 2.09 1.76	2.36 2.42 2.43 2.18 2.51	....
C. H. Jones, Agricultural Experiment Station, Vermont	3.86	.32	3.54	1.74	2.47	2.29
F. M. Hollister, Agricultural Experiment Station, Vermont	3.91	.29	3.62	1.70	2.35	2.38
L. A. Hill, Agricultural Experiment Station, New Hampshire	3.84	.19 .19	3.65	1.75 1.75	2.25 2.26	2.27 2.30
W. H. Scherffius, Agricultural Experiment Station, Kentucky	[i]3.84	.16 .25 .21	3.63	1.30 1.44 1.26	....	....
F. B. Carpenter, Virginia-Carolina Chemical Co	3.81	.....	.....	.....	.....	.....

[a] Calculated by subtracting average of insoluble nitrogen from average total nitrogen.
[b] 100 cc solution, digested 30 minutes, distilled.
[c] 150 cc solution, digested 30 minutes, distilled 100 cc.
[d] 150 cc solution, no digestion, distilled 100 cc.
[e] Total nitrogen by Gunning method; digested 4 hours.
[f] Digested 5 hours.
[g] Digested 1½ hours.
[h] Digested 2 hours.
[i] Total digested 5 hours by Kjeldahl method.

The parallel determinations of total nitrogen by the individual analysts were in all cases very close and were, therefore, averaged in the table. The average results of eight analysts using the neutral method, compared with those of eight analysts using the first and second modifications of the alkaline method, are as follows:

TABLE III.—*Total nitrogen recovered as available.*

Method.	Blood.	Cotton-seed meal.
	Per cent.	*Per cent.*
Neutral	86.5	91.6
Alkaline (1)	63.4	46.1
Alkaline (2)	69.4	58.1

Several of the analysts tried other modifications. Four made comparisons of digested and undigested charges using 100 cc of the alkaline solution. The digested

samples were heated for 30 minutes before distilling, and the undigested ones were distilled without the preliminary heating. Their results follow:

TABLE IV.—*Available nitrogen recovered by modified alkaline method.*

Analyst.	No. 1, blood.		No. 2, cotton seed.	
	Digested.	Undigested.	Digested.	Undigested.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
F. A. Urner	2.64	2.84	1.89	1.93
	2.65	2.93	1.82	1.94
C. H. Jones	2.79	2.68	1.74	1.68
F. M. Hollister	2.80	2.60	1.70	1.64
A. B. Foster	3.04	2.92	1.77	1.42
	2.96	2.88	1.86	1.60

Mr. Foster also tried the following modification: Use 150 cc alkaline solution, digest 30 minutes and distill 100 cc, running in water through a tap funnel at the same rate as the distillate comes over, thus keeping the volume of liquid in the flask nearly constant. The available nitrogen recovered in sample No. 1, blood, was 2.55, 2.56; in No. 2, cotton seed, 1.68, 1.69.

The department of foods and feeding of the Massachusetts experiment station reported results with the use of 200 cc of alkaline solution, digestion for one hour and distillation of 100 cc. The available nitrogen recovered in sample No. 1, blood, was 2.88 per cent, 3.06 per cent; in No. 2, cotton seed, 2.33 per cent, 2.32 per cent.

Mr. Sheib sent some interesting results showing the variations due to different amounts of distillate and solution in the alkaline method. His results are given below:

TABLE V.—*Results obtained by varying amounts of distillate and solution in alkaline method.*

Sample No. 1.			Sample No. 2.		
Quantity of solution employed.	Final volume of distillate.[a]	Available nitrogen.	Quantity of solution employed.	Final volume of distillate.[a]	Available nitrogen.
cc	*cc*	*Per cent.*	*cc*	*cc*	*Per cent.*
200	190	2.67	250	225	2.86
200	190	2.91	200	185	2.96
150	140	2.89	200	190	2.93
150	100	2.66	150	100	2.42
150	110	2.90	150	100	2.36
150	100	2.59	150	100	2.43
150	145	3.11	150	100	2.18
150	140	3.06	150	100	2.51
100	95	2.82	150	130	2.47
100	90	2.65	100	95	1.67
100	90	2.66	100	95	2.14
			100	95	1.87
			100	95	2.09
			100	90	1.76

[a] Approximate.

The following notes were made from the letters of the different analysts:

Department of Foods and Feeding, Amherst, Mass.—Attention is again called to the use of sulphate of potash in the plain Kjeldahl method, as it insures more perfect and

more rapid oxidation. It increases the percentage of nitrogen slightly and decreases the time of digestion to relative clearness about one-half. In the neutral permanganate method the filtrate from No. 1 shows a slight excess of permanganate and none in No. 2. The immediate distillation with the alkaline permanganate was not tried, as too much time is required for the reaction.

A. B. Foster, Maryland.—In the alkaline permanganate method using 100 cc and distilling directly until as much as possible had distilled over, the flasks containing the cotton-seed meal were found to froth badly. The last 5 cc that came over was stronger than centi-normal ammonia solution. It was also observed that it made considerable difference whether the distillation took place rapidly or slowly; the more slowly it was boiled the more ammonia distilled over.

C. H. Jones, Vermont.—The total nitrogen charges were digested from two and one-half to three hours. In the alkaline permanganate method, when the preliminary digestion is omitted, frothing is apt to occur. This is not so troublesome with the samples here reported as with garbage tankage and other low-grade materials.

F. A. Urner, New York.—For total nitrogen 1 gram was taken as a charge. It was allowed to stand over night with 20 cc sulphuric acid. In the morning it was digested slowly for one hour and then boiled for one and one-half hours. With the alkaline permanganate method, in both samples when only 100 cc of solution were used, results were low, and these were thought to be due to the fact that only 75 cc of distillate could be recovered.

C. D. Howard, associate chemist of the West Virginia station, in a communication gave the following experience with the neutral method for available nitrogen in the practical work of the fertilizer control: With ordinary medium grade fertilizers the use of a charge equivalent to 0.075 gram of nitrogen did not seem uniformly practicable, as the quantity of material represented is usually considerably more than sufficient to exhaust the 125 cc of permanganate solution. Consequently in all mixed goods a uniform charge of 2 grams was used. It was found that in this way much more concordant results could be obtained. The majority of brands met with at that station are made up on a guarantee of 0.82 per cent of nitrogen. According to the method as laid down, it would require over 9 grams for a charge, an amount which would include more organic matter in itself than would be required to exhaust the permanganate.

Recommendations.

From the data and observations obtained this year, I recommend that the first part of the neutral method be changed to read as follows: Into a 300 cc low-form Griffin beaker weigh 2 grams of material, if the sample is a mixed fertilizer; if it is from concentrated goods, use an amount containing approximately 0.075 gram of nitrogen.

I also recommend that the study of methods for the determination of available organic nitrogen be continued.

Mr. Carpenter. I have a paper on this subject prepared by Mr. Sheib to present to the association:

THE ELECTROLYTIC REDUCTION OF NITROGEN IN METALLIC NITRATES.

By S. H. Sheib.

William H. Easton, in the Journal of the American Chemical Society,[a] describes "The reduction of nitric acid in metallic nitrates to ammonia by the electric current." The results published, however, vary on a sample of chemically pure potassium nitrate between 13.32 per cent and 13.77 per cent, theory requiring 13.86 per cent.

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[a] Vol. 25, No. 10, October, 1903.

In the analysis of potassium nitrate Easton employed 0.5 gram each of potassium nitrate and copper sulphate under the following conditions: Cathode, platinum or copper plate, 100 sq. cm area; ampères, 0.15 to 3; volts, 3 to 8; dilution, 150 cc; sulphuric acid (sp. gr. 1.062), 30 cc; time, for 3 ampères, $1\frac{1}{4}$ hours; for 0.15 ampère, $8\frac{1}{2}$ hours. The solution was electrolyzed until the copper was completely deposited, after which the ammonia was determined by distillation in the usual manner.

Owing to the many objections to existing methods the electrolytic reduction as described by Easton was tried, but the results were far from satisfactory. A dried sample of sodium nitrate chemically pure, theoretically containing 16.47 per cent of nitrogen, yielded from 15.96 per cent to 16.22 per cent. A sample of potassium nitrate similarly dried gave from 13.38 per cent to 13.68 per cent (theory 13.86 per cent). These results agree fairly well with those obtained by Easton, but are still decidedly unsatisfactory. The liquid after reduction of all the copper gave, in most instances, a decided reaction with brucine, and it was hence deemed advisable to increase the proportion of copper sulphate. Further, since the odor of nitric acid was perceptible during the progress of the reduction, particularly at the start, the quantity of sulphuric acid (30 cc, sp. gr. 1.062) was reduced to 20 cc. It would seem that this quantity might be still further reduced without danger of loss of ammonia, or without materially affecting the conductivity of the solution, since the acid set free by the decomposition of the copper sulphate continually replaces the portion combining with the reduced ammonia.

Easton says: "In no trial did the variation of the current density at the anode have any influence on the amount of nitrogen reduced to ammonia." The experiments of the writer have not borne this out, but have rather tended to confirm the statement of Vortmann: "It is advantageous to use a feeble current (1 to 2 cc oxyhydrogen gas per minute)."

With currents of 1 ampère or over, the results were invariably low, the error increasing with the strength of the current, while the employment of currents of 0.3 ampère or less, gave results that were uniform, and approached theory very closely. In the examples given below, the following conditions were observed:

Temperature, that of room;
Volume of solution, 200 to 250 cc;
0.35 to 0.5 gram sodium nitrate;
1 to 2 grams copper sulphate;
20 cc sulphuric acid, sp. gr. 1.062;
Cathodes, platinum cylinders, 100 sq. cm area;
Current density, 0.15 to 0.30 ampère;
Potential, 2 to 3 volts.

The reduction was allowed to proceed over night, and the distillation and titration were performed in the usual manner.

The presence of spongy copper on the cathode seemed to exert an influence rather favorable than otherwise. While it is true that portions of the spongy deposit became detached, no effort was made to remove them from the liquid, which was distilled as usual.

Results of electrolytic reduction of nitrogen in three samples of nitrate of soda.

C. P. NITRATE OF SODA, DRIED AT 130°.

	Nitrogen.	Ammonia.	Sodium nitrate.
	Per cent.	*Per cent.*	*Per cent.*
Sample 1	16.44	19.96	99.79
Sample 2	16.48	20.02	100.04
Sample 3	16.46	19.98	99.91
Average ..	16.46	19.98	99.91
COMMERCIAL SAMPLE NITRATE OF SODA, NO. 43496.			
Sample 1	15.76	19.14	95.66
Sample 2	15.80	19.18	95.91
Average ..	15.78	19.16	[a]95.78
COMMERCIAL SAMPLE NITRATE OF SODA, NO. 43698.			
Sample 1	15.88	19.28	96.39
Sample 2	15.84	19.22	96.15
Sample 3	15.88	19.28	96.39
Average ..	15.86	19.26	96.31

[a] By method of difference, 96.03 per cent sodium nitrate.

Mr. HOPKINS. I would like to ask the referee if either of these methods for the determination of available nitrogen has been applied to materials which are pretty well known in practice to be readily available and to others not equally available, such as leather and dried blood, for example; have comparisons been made with those materials?

Mr. MORSE. These comparisons have been made by previous referees; and as regards the effect on materials, the neutral method was found to agree with simultaneous vegetation experiments which the referees carried on and with vegetation experiments conducted at the Connecticut Agricultural Experiment Station.

As to the alkaline method, no one claims anything for it except that it sorts out the poor nitrogenous material from the good, but vegetable nitrogenous material, such as cotton-seed meal, always gives low results. Therefore the trouble with the alkaline method is that it needs a check on any low result. It is claimed, however, by those who are using it right along in the routine work of fertilizer analysis that they can rapidly sort out the good nitrogen from the bad and then check the bad with some other method, so that the better class of the vegetable nitrogenous material can be distinguished.

Mr. HUSTON. I have noted with some interest the comparison or analogy drawn between the question of taking a fixed quantity of the sample, an arbitrary fixed quantity, and our practice in connection

with insoluble phosphoric acid. I suppose that quantity in connection with insoluble phosphoric acid is so fixed now in practice that it will be difficult and perhaps impossible to change it. Yet, with two or three products the method for insoluble phosphoric acid is not right at all, simply because these products contain from 35 to 50 per cent of citrate soluble phosphoric acid, and the solvent power of 100 cc of the official citrate solution is not great enough to hold up that much phosphoric acid. I thought it might be well to mention in this connection that you might get into the same sort of trouble if you adopt for the nitrogen work a single fixed quantity. It seems to me that you will be on the safe side if you have a somewhat flexible quantity, and it is easier to consider the point now than it will be after you have adopted a fixed quantity.

The case seems not unlike that of basic slag, in which the free lime in a slag neutralizes a considerable quantity of citric acid before any citric acid is available for dissolving the phosphate. So possibly some of the weak acids, or other organic matter, are really using up the permanganate solution before it gets a chance at the nitrogenous products, and just as a definite quantity of citric acid is allowed for free lime in slag so allowance may be made for this nonnitrogenous matter irrespective of the nitrogen contained in the sample.

Mr. Morse. I tried to provide in a measure for just that point. As Mr. Huston has said, we recognize that we have had much difficulty with the ammonium-citrate method and have tried to obviate that difficulty in mixed fertilizers and materials by using a fixed amount of nitrogen in the latter case and a fixed quantity of sample in the former.

The President. The next subject is the separation of nitrogenous bodies.

Mr. Van Slyke. A letter from Mr. Harcourt states that owing to illness and death in his family he has not been able to put his results on vegetable proteids together for publication and he suggests that he be allowed to finish his report, which by the way is a compilation, and forward it later for publication. This can be acted upon later.

The subject of my own work was the separation of nitrogenous bodies in dairy products.

REPORT ON SEPARATION OF NITROGENOUS BODIES IN MILK AND CHEESE.

By L. L. Van Slyke, *Referee.*

In the referee's report last year the writer described, among other methods, one devised by Mr. E. B. Hart and himself for the determination of unsaturated paracasein lactate, that is, paracasein monolactate, in cheese. Since in normal American cheddar cheese we have at the start only paracasein and paracasein monolactate, the separation of these compounds is readily affected by the method given; that is, by dissolving the paracasein monolactate in a 5 per cent solution of sodium chlorid. Under rare and abnormal conditions we may possibly have a mixture of paracasein, paracasein monolactate, and paracasein dilactate. Since we have not yet been com-

pelled to face a condition of this kind, no provision has been made for such an emergency, and at present no satisfactory method can be given for the separation of paracasein and paracasein dilactate.

Mr. Hart and I have been giving our attention recently to the study of the corresponding casein salts, casein monolactate and casein dilactate, and their formation in the ordinary souring of milk has been studied. Some study has been made also of the relation of these compounds to the determination of casein in milk. While this work is not yet completed, it is deemed desirable to report progress at this time.

I. Casein Monolactate and Casein Dilactate.

1. Determination of casein monolactate in milk.

Casein monolactate in milk coagulates readily at 40° C. Hence, in a milk containing only casein and casein monolactate, the monolactate can be separated by heating the milk to about 40° C., filtering the precipitate found, washing, and determining the nitrogen in the precipitate. Ten grams of milk diluted with 90 cc of water give good results.

2. Separation of casein monolactate and casein dilactate.

Casein dilactate coagulates completely at 40° C. and below. In milk containing casein monolactate and dilactate, heat 10 grams of milk diluted with 90 cc of water to 40° C., and these two salts precipitate and are separated from the milk casein by filtration. The washed precipitate is then treated with 100 cc of a 5 per cent solution of sodium chlorid and the whole heated to 55° C. with frequent agitation for two hours. The process is facilitated somewhat by the presence of pure quartz sand. The casein monolactate goes into solution and is separated from the casein dilactate by filtration and washing.

3. Separation and determination of casein; casein monolactate and casein dilactate in milk.

(*a*) The total amount of nitrogen precipitated by acid is determined by the official method prescribed for determining casein in milk.

(*b*) Heat 10 grams of milk diluted with 90 cc of water to 40° C. for fifteen or twenty minutes, filter the precipitate formed, and wash with distilled water. The precipitate is then transferred to a small Erlenmeyer flask provided with a stopper, treated with 100 cc of a 5 per cent solution of sodium chlorid, and heated at 55° C. for two hours, with frequent agitation. The mixture is then filtered, the remaining precipitate washed with water, and the nitrogen determined in both the precipitate and filtrate. The nitrogen in the precipitate represents casein dilactate; that in the filtrate, casein monolactate. The sum of these two subtracted from the total nitrogen found by precipitation with acid gives the amount of nitrogen as casein.

II. The Official Method for Determination of Casein in Cow's Milk.

Ten years ago, when I first submitted to this association the present official method for the determination of casein in cow's milk, the question suggested itself as to what the precipitating action of acid was upon casein and whether there was a chemical union of acid and casein. In the light of the recent discoveries referred to above, this query may now be answered. When 10 grams of cow's milk is treated with 1.5 cc of 10 per cent acetic acid, the precipitate formed is wholly casein diacetate. Some results of our work, not yet completed, show that much less acid than this is required to form the diacetate. It is probable that we shall find it desirable to reduce the amount of acetic acid used to 1 cc or less.

In this connection a fact to which the writer called attention some years ago can

now be explained. In the case of most milks it was found that if the filtrate from the acetic acid precipitate of casein was neutralized, we obtained more or less precipitate, usually about one-tenth of 1 per cent of casein. The suggestion offered was that this neutralization precipitate was due to solution of the casein precipitate in excess of acid. We now know that this explanation is true, and why. Saturated salts formed by casein and acids dissolve quite easily in excess of free acid. With the interpretation we give of the action of acids upon casein, there is good reason to believe that our method can be so modified as to avoid this error and also avoid the additional determination of a neutralization precipitate. Another year we shall be able to report the results of a completed investigation in this line.

III. Relation of Casein Monolactate and Casein Dilactate to the Hess and Doolittle Method of Detecting "Renovated" Butter.

This test is based upon the difference alleged to exist between the characteristics of the curd derived from pure butter and that derived from renovated butter. According to the statement of Hess and Doolittle, "the curd from true butter will have an amorphous, nongranular appearance, while the curd from "process" butter has a very coarse curdy appearance. The one is the proteid of cream; the other is the proteid of milk; the one is a gelatinous, ropy mass; the other is a granular, easily divided substance." In respect to chemical characteristics, Hess and Doolittle state that the fat-free curd and brine obtained from genuine butter give only a slight milkiness when treated with acetic acid, while the filtrate from "renovated" butter gives an abundant white precipitate. Mr. Patrick presented an exhaustive study of this test at our meeting two years ago. He spoke of the proteid of butter serum which coagulates on addition of acetic acid as "soluble casein," and found that the test did not suffice to distinguish between genuine butter and "process" butter.

Upon considering the process of butter making, it is readily understood that in the course of cream ripening lactic acid is formed and this unites with casein. If the ripening is slight—that is, if only a moderate amount of acid is developed, only casein monolactate is formed; if the cream is allowed to become sourer, a mixture of casein monolactate and dilactate or only dilactate is formed. Suppose only casein monolactate is formed, then the curd in the butter is casein monolactate. When such butter is melted the salt dissolves and with the casein monolactate falls to the bottom of the vessel. The salt brine dissolves the casein monolactate, which readily precipitates on addition of acetic acid. Moreover, the description of curd given by Hess and Doolittle as a gelatinous, ropy mass applies exactly to the brine-dissolved casein monolactate. What they describe as a "granular, easily divided substance," is very clearly casein dilactate. It should be stated that frequently, even when the casein in butter is present as dilactate, the curd suspended in the brine appears to be in a very finely divided condition, caused undoubtedly by the agitation produced in churning. Another point is that casein dilactate appears to be somewhat soluble in salt brine of a concentration such as we find in the serum of salted butter. Under these circumstances, if the butter serum is filtered the filtrate usually will give a precipitate on the addition of acetic acid.

Another point of interest stated by Mr. Patrick is the gradual disappearance of "soluble casein" in butter. In other words, fresh butter containing only casein monolactate may form enough acid from the occluded milk sugar to convert the casein monolactate into casein dilactate. Prolonged action of salt on the proteid of the butter in many instances may also account for the presence of coarser particles of curd in older butters.

It can therefore readily be seen, in the light of the existence of two such compounds as casein monolactate and casein dilactate, that it is a mere question of the amount of acid formed in the ripening of cream or milk as to whether one set of char-

acteristics or another will be present. We can make genuine butter that will respond to the Hess and Doolittle test for "process" butter, or vice versa. Moreover, where a fresh, genuine butter responds to the test for pure butter, the same butter, if kept some weeks, responds to the test for a "process" butter. Such a test must be regarded as unreliable, and there does not at present seem to be much hope of finding a method of distinguishing between genuine butter and "process" butter on the principle proposed by the Hess and Doolittle test.

Recommendations.

Your referee suggests the following recommendations:

1. That the method given for the separation and determination of casein monolactate and casein dilactate be adopted as a provisional method.
2. That Mr. Harcourt's report on vegetable proteids be included in the printed proceedings.

REPORT ON THE SEPARATION OF VEGETABLE PROTEIDS.

By R. Harcourt, *Associate Referee.*

Your referee found it impossible to do any extended investigation work on the separation of the vegetable proteid bodies and has confined his attention more to reviewing the literature on this subject. This report is, therefore, largely a compilation of the results obtained by those who have given this matter particular study. Many quotations are from the findings of Osborne and his coworkers.

Under the generic name of proteids are classed a number of highly complex compounds. As a class they are white or yellowish nonvolatile solids, usually, but not invariably, amorphous. They are unchangeable in the dry condition, but putrify readily when moist or in solution. Some of the proteids are soluble in water, others in neutral salt solutions, and some in alcohol, while they appear to be invariably insoluble in ether, chloroform, and carbon disulphid. They are mostly precipitated from their solutions by a great number of reagents, including certain salts of the light metals, salts and hydroxids of the heavy metals, mineral acids, certain organic acids, etc. According to Osborne,[a] the proteids are basic bodies and enter into some reactions with acids, with which they form true salts. They are decomposed by treatment with strong mineral acids, ammonia, leucin, tyrosin, and various other bodies being products of the decomposition. The amid compounds, of which asparagin and leucin are types, coexist in the plant with the higher proteids, and are probably intermediate compounds in the formation of the proteids. They occur in all living matter, being essential constituents of the protoplasm.

The various proteids differ somewhat in elementary composition. Hoppe-Seyler[b] gives the following limits:

Limits in composition of proteids (Hoppe-Seyler).

Data.	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Minimum	51.5	6.9	15.2	0.3	20.9
Maximum	54.5	7.3	17.0	2.0	23.5

Osborne's analyses of at least two globulins, legumin, and edestin, indicate that the limit for nitrogen is not wide enough, for both these substances contained over 18 per cent of nitrogen.

While the proteids do not vary widely in composition, there is sufficient difference in their general properties to allow them to be arranged in groups.

[a] J. Amer. Chem. Soc., **24**: 76.

[b] Watt's Dictionary of Chemistry, **4**: 327.

(*a*) *Vegetable albumens* have been found in the cereal grains. They are soluble in water, and coagulate at 70°. Sodium chlorid, magnesium sulphate, and acetic acid fail to precipitate them from their solutions.

(*b*) *Vegetable globulins* appear to be of very wide occurrence. They form the greater part of the proteids of the grains of the legumes and are found in smaller quantities in the cereal. They are insoluble in pure water and soluble in sodium chlorid solutions, from which they are precipitated by separating the salt from their solutions by dialysis. Coagulation takes place when their solutions are heated to 55° to 75°.

(*c*) *Vegetable albuminates.* It is now thought that the albuminates do not exist as such in plants, but that they are produced by the action of the reagents employed in the separation of the albumens and globulins. The researches of Hoppe-Seyler, Vines, Green, Martin, Osborne, and others have shown that the legumin and conglutin separated by Braconnot and Ritthausen are not albuminates, but true globulins. Osborne and Campbell[a] have shown that minute quantities of acid materially affect the solubility of globulins in salt solutions.

(*d*) *Vegetable proteoses* are found in the aleurone grains. These substances are intermediate in the formation of peptones from the higher proteids. They differ very slightly from the peptones, being, like them, soluble and slightly diffusible.

(*e*) *Vegetable peptones.*—True peptones apparently do not exist as such in the plant except transiently. They are probably formed by the action of ferments upon the proteids, and as peptones are very soluble in water and diffusible, they may thus pass to the growing portion of the organism.

(*f*) *Insoluble vegetable proteids* have an undoubted existence, in the sense that they can not be dissolved out of the vegetable tissues by solvents not acting chemically on them; but it is probable that such insoluble proteids do not preexist in the plant, but are produced by the action of ferments similar to those which occasion the coagulation of blood and milk. "The most important number of the group is gluten, a tenacious, tasteless, brownish-gray substance. It consists of two substances, one soluble in alcohol, which has been called 'gliadin' and another portion insoluble in alcohol, but soluble in weak alkali, called 'glutenin'".[b] In contradiction to this Osborne[c] states: "No ferment action occurs in the formation of gluten, for its constituents are found in the flour having the same properties and composition as in the gluten."

A vast amount of work has been done on the separation of these various classes and of individual proteid substances. Newer and more delicate methods of analysis have greatly aided this work and have exposed many of the errors of the earlier investigations. In this report I will not dwell on the conclusions of the earlier investigators, but shall seek to give the results of the latest work on some of the more important food products.

Proteids of Wheat.

Leibig, Boussingault, Dumas, De Saussure, and many others have worked on the proteids of wheat. The latest and most complete work is reported by Osborne and Voorhees.[d] According to these investigators there are five proteids in wheat. The following is their summing up of the investigations of the wheat kernel:

The proteids contained in the wheat kernel are:

I. A globulin belonging to the class of vegetable vitellins, soluble in saline solutions, precipitated therefrom by dilution and also saturation with magnesium sulphate or ammonia sulphate, but not by saturation with sodium chlorid. Partly precipitated by boiling, but not coagulate at temperatures below 100°. The wheat

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[a] J. Amer. Chem. Soc., **19**: 482.

[b] Ingle, Manual of Agricultural Chemistry, p. 214.

[c] Amer. Chem. J., **15**: 471.

[d] Amer. Chem. J., **15**: 392.

kernel contains between 0.6 and 0.7 per cent of this globulin. When dried at 110° its composition was found to be as follows:

Wheat globulin.

	Per cent.
Carbon	51.03
Hydrogen	6.85
Nitrogen	18.39
Sulphur	.69
Oxygen	23.04
	100.00

II. An albumin, coagulating at 52°, which differs from animal albumin in being precipitated on saturating its solutions with sodium chlorid or with magnesium sulphate, but not precipitated on completely removing salts by dialysis in distilled water. It was found to form between 0.3 and 0.4 per cent of the wheat kernel, and to have the following composition when separated from solution in the coagulated form by heating to 60° C.:

Wheat albumin.

	Per cent.
Carbon	53.02
Hydrogen	6.84
Nitrogen	16.80
Sulphur	1.28
Oxygen	22.06
	100.00

III. A proteose, precipitated (after removing the globulin by dialysis and the albumin by coagulation) by saturating the solution with sodium chlorid, or by adding 20 per cent of sodium chlorid and acidifying with acetic acid. This body was not analysed in its unaltered form. On concentrating its solutions by boiling, a coagulum was gradually developed which formed about 0.3 per cent of the wheat kernel and had the following composition:

Coagulum.

	Per cent.
Carbon	51.86
Hydrogen	6.82
Nitrogen	17.32
Sulphur, Oxygen	24.00
	100.00

The solution filtered from the substance just described still contained a proteose-like body which was not obtainable in a pure state. Its amount could only be roughly estimated by precipitating the concentrated filtrate from the preceding substance with alcohol and multiplying the nitrogen contained in the precipitate by 6.25. The amount of this proteose was from 0.2 to 0.4 per cent of the seed. Both these substances are unquestionably derivatives of some other proteid in the seed, presumably the proteose first mentioned.

IV. Gliadin, soluble in dilute alcohol and forming about 4.25 per cent of the seed. It has the following composition:

Gliadin.

	Per cent.
Carbon	52.72
Hydrogen	6.86
Nitrogen	17.66
Sulphur	1.14
Oxygen	21.62
	100.00

This is the proteid called gliadin by Taddei and plant gelatin by Dumas and Cahours. Mixed with impurities or altered to a greater or less extent by the process of separation, it has been described by Ritthausen under the name of gluten

fibrin, plant gelatin, or gliadin, and mucedin, and by Martin has been termed insoluble phytalbumose. The mucin of Berzelius and of De Saussure were impure preparations consisting chiefly of this proteid. It is soluble in distilled water to opalescent solutions which are precipitated by adding a little sodium chlorid. It is completely insoluble in absolute alcohol, but slightly soluble in 90 per cent alcohol, and very soluble in 70 to 80 per cent alcohol and is precipitated from these solutions on adding either much water or strong alcohol, especially in the presence of much salts; soluble in very dilute acids and alkalies, and precipitated from these solutions by neutralization, unchanged in properties and composition. This proteid is one on which the formation of gluten largely depends.

V. Glutenin, a proteid insoluble in water, saline solutions and dilute alcohol, which forms the remainder of the proteids of the wheat kernel, generally about 4 to 4.5 per cent of the seed. This substance is soluble in dilute acids and alkalies and is precipitated from such solutions by neutralization. Dissolved in 0.2 per cent potash water, precipitated by neutralization and, after thorough extraction with alcohol and either, again dissolved in potash water, the solution filtered clear, precipitated by neutralization and dried at 110°, it has the following composition:

Glutenin.

	Per cent.
Carbon	52.34
Hydrogen	6.83
Nitrogen	17.49
Sulphur	1.08
Oxygen	22.26
	100.00

Unless prepared as above described, the impurities are not removed and the analyses are discordant. This proteid was first described by Taddei under the name of zymon. Liebig as well as Dumas and Cahours named it plant fibrin. Ritthausen called it gluten casein, Weyl and Bischoff considered it to be an albuminate form of a myosin-like globulin. Martin named it gluten fibrin and likewise considered it to be an albuminate form of a myosin-like globulin.

VI. Wheat gluten is composed of gliadin and glutenin. Both these proteids are necessary for its formation. The gliadin with water forms a sticky medium, which by the presence of salts is prevented from becoming wholly soluble. This medium binds together the particles of flour, rendering the dough and gluten tough and coherent. The glutenin imparts solidity to the gluten, evidently forming a nucleus to which the gliadin adheres and from which it is consequently not washed away by water. Gliadin and starch mixed in the proportion of 1:10 form a dough, but yield no gluten, the gliadin being washed away with the starch. The flour freed from gliadin gives no gluten, there being no binding material to hold the particles together so that they may be brought into a coherent mass.

Soluble salts are also necessary in forming gluten, as in distilled water gliadin is readily soluble. In water containing salts it forms a very viscid, semifluid mass, which has great power to bind together the particles of flour. The mineral constituents of the seeds are sufficient to accomplish this purpose, for gluten can be obtained by washing a dough with distilled water.

VII. No ferment action occurs in the formation of gluten; for its constituents are found in the flour, having the same properties and composition as in the gluten, even under those conditions which would be supposed completely to remove antecedent proteids or to prevent ferment action. All the phenomena which have been attributed to ferment action are explained by the properties of the proteids themselves as they exist in the seed and in the gluten.

Proteids of Rye.

The proteids of this seed have not been as exhaustively studied as those of the wheat. Einhof, Heldt, Ritthausen, and Von Bibra succeeded in separating proteids similar to the gliadin and glutenin of wheat. Verdeil[a] claimed to have washed gluten out of rye flour, but did not consider it pure because it was contained with a

[a] Ann. d. Chem. u. Pharm., 58: 319.

substance soluble in alcohol. The following are the conclusions arrived at by Osborne after his study of the rye kernel:[a]

Owing to the gum already mentioned the filtration and treatment of the rye extracts was difficult and prolonged and the amounts of globulin, albumin, and proteose could not be determined separately, as in the case of wheat. The rye flour contained 1.52 per cent of nitrogen. If we assume that the proteids of rye contain on the average 17.6 per cent of nitrogen, as was very nearly the case with those of wheat, and that all the nitrogen exists in proteid form, this sample of flour would contain 8.63 per cent of proteid. We have, therefore, 2.44 per cent of insoluble proteid and 6.19 per cent soluble in salt solution and alcohol.

We have already shown that the alcohol-soluble gliadin amounted to 4 per cent of the flour and the leucosin to 0.43 per cent. There thus remains 1.76 per cent to be divided between edestin and proteoses.

	Per cent.
Insoluble in salt solution	2.44
Gliadin, soluble in alcohol	4.00
Leucosin, soluble in water	0.43
Edestin and proteose, soluble in salt solution	1.76
	8.63

Proteids of Barley.

Even less attention has been paid to the study of the proteids of barley than to those of rye. The conclusions reached by Osborne after an exhaustive study of the proteids of barley are as follows:[b]

The barley kernel contains—

I. Leucosin coagulating at 52°, which is the same as the albumin found in the wheat and rye kernels. Its composition, as shown by the average of six analyses, is:

	Per cent.
Carbon	52.81
Hydrogen	6.78
Nitrogen	16.62
Sulphur	1.47
Oxygen	22.32
	100.00

This substance forms about 0.3 per cent of the seed.

II. A small quantity of proteose, the reactions and composition of which could not be definitely ascertained.

III. Edestin, a globulin which is the same as that found in the wheat and rye kernels and in a large number of other seeds. Its composition is approximately shown by the figures given below. Owing to the small amount of this body and the difficulty in preparing it, no perfectly pure preparations were obtained.

	Per cent.
Carbon	50.88
Hydrogen	6.65
Nitrogen	18.10
Sulphur, Oxygen	24.37
	100.00

This is the proteid commonly known as vegetable vitellin. It is precipitated from saline solutions by dilution and by dialysis, is not coagulated by heating below 90°, and above that temperature only partially. It is not precipitated by saturating its solution with sodium chlorid, but is thrown down from saline solutions by adding acid.

IV. Hordein, a proteid insoluble in saline solutions, very slightly soluble in pure water, and extremely soluble in alcohol of about 75 per cent. This is barley proteid

[a] J. Amer. Chem. Soc., 17: 447.
[b] J. Amer Chem. Soc., 17: 565.

described by Ritthausen as mucedin. It has almost the same physical and chemical properties as gliadin obtained from wheat and rye kernels but a different composition.

	Per cent.
Carbon	54.29
Hydrogen	6.80
Nitrogen	17.21
Sulphur	.83
Oxygen	20.87
	100.00

About 4 per cent of the seed consists of this substance.

V. After extracting the barley flour with salt solution and with alcohol the residue still contained 42 per cent of the total nitrogen, corresponding to proteid matter equal to about 4.5 per cent of the flour. It was not possible to extract more than a very small amount of this residual proteid with dilute potash water, as the treatment for removal of the other proteids rendered it soluble, if it were not so already.

VI. The barley flour contained 1.83 per cent of nitrogen, and if it be assumed that this all belonged to proteid matter with 17 per cent of nitrogen, the flour would contain 10.75 per cent of proteids. The barley accordingly contained about 4.5 per cent of insoluble proteid, 4 per cent of hordein soluble in dilute alcohol, 0.3 per cent of albumin, and 1.95 per cent of globulin and proteose.

The Proteids of the Pea, Lentil, Horse Bean, and Vetch.

The proteids of the leguminous seeds appear to be very much alike in their reactions and properties, though differing widely in the quantity of the various proteids found in the grains of this interesting group. Osborne and Campbell have summed up their properties as follows:[a]

LEGUMIN.

Legumin forms the chief proteid constituent obtainable from the vetch, pea, lentil, and horse bean. In the first-named seed about 10 per cent of the meal was found to consist of legumin; in the three other seeds this proteid is associated with vicilin, from which we have no method for its quantitative separation. From the pea about 10 per cent, from the lentil 13 per cent, and from the horse bean about 17 per cent of these mixed proteids were obtained. The lentil contains the least proportion of legumin, which seems to form about two-thirds of the mixed proteids, while the horse bean contains the greatest, as in this seed vicilin is present in relatively small amount.

Legumin is a globulin, for it dissolves readily in saline solutions, and is precipitated therefrom either by dialysis, dilution, or cooling. By dialysis or by cooling it separates in the form of spheroids which, after settling from the solution, unite to form a plastic mass. By diluting its concentrated solutions the legumin separates as a viscid, translucent fluid. This fluid, when treated with water, becomes opaque and solid, so that the legumin can be converted into a coarse meal by rubbing with a glass rod under water. Conglutin from lupin seeds and amandin from almonds behave similarly, as do gliadin of wheat and rye and hordein of barley, when precipitated from alcoholic solutions by dilution with water.

Solutions containing more than 2 per cent of sodium chlorid dissolve legumin abundantly; those containing less salt have a solvent power rapidly decreasing with the diminishing salt content, so that a 1 per cent salt solution dissolves very little. Saturation with sodium chlorid or magnesium sulphate does not precipitate legumin from its solution in brine, but saturation with sodium sulphate at 34° precipitates it almost completely.

In pure water legumin is entirely insoluble, but if the solution from which the legumin is precipitated contains acid, this may combine with the legumin, and the resulting preparation, like other acid globulins, will then dissolve in pure water.

If seeds containing legumin are extracted with water, more or less of the legumin is dissolved—from the pea about 4 per cent, from the vetch 2.5 per cent, from the lentil 10 per cent, and from the horse bean 16 per cent. The legumin thus dissolved is largely precipitated by dialysis in water, by the addition of acids and by lime salts and very slightly by great dilution with water. These aqueous extracts

[a] J. Amer. Chem. Soc., **20**: 410.

react strongly acid with litmus; and alkaline with lacmoid, a behavior doubtless due to acid potassium phosphates, together with organic acids or acid salts for acids combined with proteids do not react with lacmoid, although they readily turn blue litmus red. Solutions which we have made of legumin from various seeds, as well as of edestin from hemp seeds, by dissolving the proteid in monohydrogen potassium phosphate, have shown us all the reactions of these aqueous extracts excepting one presented by the horse bean, the unneutralized water extracts of which are precipitated with pure sodium chlorid. This reaction we can not explain; for these extracts give with dilute acids precipitates soluble in salt solutions and no precipitates on neutralization, reactions which seem to exclude the presence of acid globulin.

Solutions of legumin or of edestin in monohydrogen potassium phosphate behave more like solutions in dilute alkali carbonates than like solutions in neutral salts; for they give precipitates with dilute acids which are soluble in more acid or in salt solutions and are not precipitated by dilution unless very little phosphate is present. If phosphoric acid is added to the potassium phosphate solution, the solvent power of the phosphate is diminished and the facility with which the proteid is precipitated by dilution is increased; but if only little acid is added, yet enough to give a decided reaction with litmus, considerable quantities of the globulins are still dissolved and the reactions of solutions so made closely resemble those of the aqueous extracts of these seeds. As a result of numerous comparisons of the reactions of solutions so prepared with those of the aqueous extracts of leguminous seeds, we believe that the extraction of legumin by water from these seeds is due to the presence of acid potassium salts of phosphoric and organic acids, and that in consequence of the varying proportions of these substances in the different kinds of seeds, different amounts of legumin are thus extracted from them. As the proportion of phosphoric acid to potash in these leguminous seeds is much smaller than in most of the other seeds which we have examined, the character of the salts present may fairly be supposed to differ, and consequently the solubility of the proteids would also differ when the seeds are extracted with water. Thus in lupin seeds the amount of phosphoric acid, 1.4 per cent, exceeds that of the potash, 1.1 per cent, and from them water extracts but little proteid. Liebig and Ritthausen attributed the solubility of legumin to basic phosphates, and the latter undertook extensive experiments to show the presence of an excess of potash in the aqueous extracts; but such a supposition appears to overlook the fact that the aqueous extracts of these seeds are decidedly acid toward litmus.

Dissolved in dilute sodium chlorid solution, legumin is precipitated by a little acetic acid, the precipitate being soluble in an excess of sodium chlorid. The solubility of precipitates so produced depends on the relative proportions of salt and acid.

Legumin extracted without neutralizing the natural acid of the seed precipitated by dialysis, either directly or after precipitation with ammonium sulphate, is usually converted to a large extent into insoluble "albuminate."

This "albuminate" differs from the similar insoluble products obtained from most other globulins; for when treated with salt solution it becomes gelatinous, can not be filtered, and, on washing with water, shrinks, becomes opaque, and finally granular, so that it can be very readily washed on a filter. This substance appears to become hydrated by salt solution and dehydrated by pure water.

If the acid of the seed is previously neutralized, the globulin extracted by salt solution yields very little if any insoluble "albuminate," which indicates that the latter is a product of the action of the acid of the seed. This fact is in harmony with experiments described in a former paper of ours on the action of minute quantities of acid on globulins. In very dilute acids, and alkalies in absence of salts, legumin dissolves readily and abundantly, from which solutions, if at once neutralized, it is precipitated in a form soluble in sodium chlorid solution. By this treatment no evidence of change has been detected. Solutions made with hydrochloric acid are precipitated by a small excess of acid, but those made with acetic acid are not precipitated by any excess of acid.

Solutions of legumin in 10 per cent sodium-chlorid brine are not rendered turbid by long heating in a boiling water bath.

Dissolved in 10 per cent sodium-chlorid brine, legumin is precipitated by a very little hydrochloric acid, but a relatively considerable amount of acetic acid is required to produce a precipitate in such solutions.

Sodium-chlorid solutions of legumin give large precipitates with tannic acid, as well as with picric acid, those formed by the latter dissolving in an excess of salt solution if too much picric acid had not been previously added. With mercuric chlorid no precipitate is produced.

With nitric acid, Milton's and Adamkiewicz's tests, proteid reactions are obtained. With the biuret test a violet color is given, which on standing becomes a deep rose-red like that given by peptones.

Legumin has been supposed by some investigators to contain phosphorus, and therefore to belong with the nucleo-proteids. A careful testing of thoroughly purified samples, by fusing with caustic soda and nitrate, and treating the solution of the fusion acidified with nitric acid with ammonium molybdate, showed that in some of the preparations only just detectable traces of phosphorus were present, while other preparations contained none whatever.

Although we have examined large numbers of our preparations of the different plant proteids for phosphorus, we have as yet found none which in carefully purified samples contained more than a few hundredths of a per cent, a quantity so small that it is reasonable to consider it as a constituent of the ever-present ash.

The composition of legumin is shown by the following figures, which are averages of a number of preparations from each of the different seeds:

Legumin.

Constituents.	Pea.	Lentil.	Horse bean.	Vetch.	Average.
Carbon	51.74	51.73	51.72	51.69	51.72
Hydrogen	6.90	6.89	7.01	6.99	6.95
Nitrogen	18.04	18.06	18.06	18.02	18.04
Sulphur	.42	.42	.39	.43	.41
Oxygen	22.90	22.92	22.82	22.87	22.88
Total	100.00	100.00	100.00	100.00	100.00

VICILIN.

Vicilin is a globulin associated with legumin in the pea, lentil, and horse bean. But as we have no means of separating vicilin and legumin quantitatively, we can state nothing respecting the amount in which it occurs in these seeds further than that the lentil contains the most and the horse bean the least. In the lentil it probably forms about one-third of the mixed globulins. That vicilin is not derivative of legumin, is almost conclusively proved by the fact that no vicilin can be obtained from the vetch.

The most remarkable characteristic of vicilin is its content of sulphur, less than that of any other known proteid. This element, it may be noticed, diminishes in quantity with repeated precipitation, as though by this process sulphur were split off from the molecule. The total quantity, however, is so small that it might appear unsafe to draw conclusions from the differences observed; that is, from 0.23 per cent maximum to 0.08 per cent minimum. Yet, repeated determinations of this element have shown us that the differences were not analytical. It would seem possible by sufficiently repeated precipitation to obtain from this preparations free from sulphur.

In salt solution vicilin is much more soluble than legumin, so that by repeated precipitation from diluted solutions the two globulins can be separated.

When solutions of vicilin in 10 per cent brine are heated in a water bath they become turbid at 90°, and at 95° flocks separate. When heated for some time at 100° this globulin is almost completely coagulated. In water vicilin is insoluble. In 1 per cent sodium chlorid solution it dissolves considerably, while in slightly stronger solutions it is much more soluble, the solution of the globulin appearing to depend on the presence of enough salt to form a soluble compound.

In its reactions it very closely resembles legumin.

The composition of vicilin we found to be as follows:

Vicilin.

Constituents.	Pea.	Lentil.	Horse bean.	Average.
Carbon	52.36	52.13	52.38	52.29
Hydrogen	7.03	7.02	7.04	7.03
Nitrogen	17.40	17.38	17.52	17.43
Sulphur	.18	.17	.15	.17
Oxygen	23.03	23.30	22.91	23.08
Total	100.00	100.00	100.00	100.00

LEGUMELIN.

We have found legumelin in all leguminous seeds which we have examined with the exception of the white bean (*Phaseolus vulgaris*) and the blue and yellow lupin.

It is difficult to decide whether this proteid should be considered an albumin or a globulin.

By prolonged dialysis of solutions containing considerable legumelin a very small part separates, usually in a form insoluble in salt solutions. In one case we obtained a precipitate by dialysis which was wholly soluble in brine, to a solution from which a relatively large coagulum separated on heating to 65°, thus indicating a globulin. We have found, however, that other proteids, notably edestin, undergo a change in solubility, whereby the proteid becomes gradually more and more difficult to dissolve. Thus edestin changes from a form insoluble in cold brine to one soluble in hot brine, also to one soluble in hot brine to one insoluble therein, but soluble in dilute sodium carbonate solution, and finally from a form insoluble in alkali carbonate to one soluble only in caustic alkali. It is not unreasonable to expect that a proteid which might properly be considered an albumin (i. e., soluble in pure water and coagulable on heating) should undergo a similar change whereby it becomes no longer soluble in pure water but dissolves in salt solutions and then further changes to a form no longer soluble in brine but dissolved by alkali carbonates.

Such a very large proportion of the legumelin resists precipitation by dialysis, that we are inclined to regard the small precipitates which we have obtained in this way as due to changes similar to those which produce so-called "albuminates" from globulins.

In a paper on the proteids of the cowpea[a] we designated this proteid as a globulin, but in view of our subsequent, much more extensive experience we now consider legumelin to be more properly classed with the albumins.

The amount of legumelin which we have found in the seeds discussed in this paper was in the pea 2 per cent, vetch, 1.5 per cent; lentil and horse bean, 1.25 per cent.

No definite coagulation point can be stated for legumelin, as the presence of salts or acids as well as the proportion of dissolved legumelin have a great effect on the temperature at which coagulation takes place. * * *

In the following table we give the average of analyses which we have made of coagulated legumelin from different seeds. These preparations have been obtained in a variety of ways, as can be seen by consulting our papers on the seeds named.

Legumelin.

Constituents.	Pea.	Lentil.	Horse bean.	Vetch.	Adzuki bean.	Cow-pea.	Soy bean.	Aver-age.
Carbon	53.31	53.22	53.03	53.31	53.97	53.25	53.06	53.31
Hydrogen	6.99	6.82	6.97	6.97	7.01	7.07	6.94	6.97
Nitrogen	16.30	16.27	16.22	16.24	16.31	16.36	16.14	16.26
Sulphur	1.06	.94	1.30	1.11	.88	1.11	1.17	1.08
Oxygen	22.34	22.75	22.48	22.37	21.83	22.21	22.69	22.38
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

PROTEOSE.

As the proteose of these seeds is present in small amount and is difficult to obtain pure, we have not as much information respecting it as is desirable. The pea appears to contain about 1 per cent, the horse bean about 0.5 per cent, and the lentil and vetch evidently less. It is probable that more or less of this proteose may be lost by diffusion, for 10.5 grams of what was doubtless nearly pure proteose from the pea after solution and dialysis yielded only about 6 grams when precipitated.

We have obtained a few of the reactions of proteose from the pea and horse bean, but no reactions of this proteid from the lentil and vetch.

[a] J. Amer. Chem. Soc., 19: 499.

Proteose.

Constituents.	Pea.		Lentil, No. 68.	Horse bean.		Vetch, No. 22.
	No. 46.	No. 47.		No. 89.	No. 98.	
Carbon	50.24	49.66	50.17	50.24	49.96	50.85
Hydrogen	6.76	6.78	6.77	6.66	6.76	6.75
Nitrogen	17.35	16.57	16.81	17.11	16.95	16.65
Sulphur	1.25	1.40	1.27	1.87	2.75	25.75
Oxygen	24.40	25.59	23.98	24.12	23.58	.....
Total	100.00	100.00	100.00	100.00	100.00	100.00

If the difficulty encountered in purifying these preparations of proteose and the different methods by which they have been obtained are considered, the agreement between them, except for the sulphur in those from the horse bean, makes it probable that these figures nearly represent the composition of this substance.

Proteids of the Maize Kernel.

The proteids of corn have been investigated by Osborne and Chittenden.[a] These proteids may be distinguished according to their solubilities as follows:

(*a*) Proteid, soluble in pure water, having some of the properties of proteose.

(*b*) Globulins, insoluble in pure water, but soluble in salt solutions. Edestin and maysin.

(*c*) Proteid, insoluble in water or salt solutions, but soluble in alcohol of 60 to 99 per cent. Zein.

(*d*) Proteid matter, insoluble in water, salt solution, and alcohol, but soluble in dilute alkalies and acids.

Later Osborne[b] determined the quantities of these proteids in corn. One hundred grams of the yellow corn meal contained, approximately, as follows:

		Grams.
Proteid soluble in 0.2 per cent potash	3.15 grams containing 15.82 per cent N.	=0.4983
Zein	5.00 grams containing 16.13 per cent N.	=0.8065
Very soluble globulin	0.04 grams containing 15.25 per cent N.	=0.0061
Edestin	0.10 grams containing 18.10 per cent N.	=0.0181
Maysin	0.25 grams containing 16.70 per cent N.	=0.0417
Proteose	0.06 grams containing 17.00 per cent N.	=0.0102
		1.3809
Nitrogen undissolved by dilute potash water		0.1645
Total		1.5454
Nitrogen in meal by analysis		1.5400
Mean percentage of nitrogen in maize proteids		16.057

The various proteids that have been separated from some other vegetable substances might be stated, but enough has been given to indicate the general characteristics of the proteids of vegetable substances. Reference has been made to the readiness with which these bodies change when in a moist condition or when they are in solution, and to the fact that they combine with acids, forming new substances with new properties. In addition to this, the solubilities of the separate proteids

[a] Amer. Chem. J., **13**: 453, 529.

[b] J. Amer. Chem. Soc., **19**: 532.

will not permit of the extraction of one proteid without being more or less contaminated with another. For general comparative purposes, however, the separation of the proteids into water-soluble, salt-soluble, alcohol-soluble, and insoluble proteids gives valuable results. The following method proposed by Prof. H. H. Snyder[a] for the separation of the proteids of flour, based largely on the methods employed by Osborne and Voorhees will apply fairly well to all vegetable substances.

Water-soluble nitrogen.—Fifteen grams of flour were weighed into a flask and 250 cc distilled water added, the temperature being kept at about 30° by placing the flask in water of that temperature. The flask was shaken occasionally. After two hours, 200 cc of the clear filtrate were placed in a Kjeldahl digestion flask containing a little sulphuric acid and evaporated nearly to dryness, when the remainder of the acid necessary for oxidation was added and the determination of nitrogen completed in the usual way. When it was also desired to separate water-soluble gliadin 30 grams of flour and 500 cc of water were used. One-half of the filtrate was evaporated nearly to dryness and the residue treated with 70 per cent alcohol to extract the soluble gliadin. The extract was filtered through a small filter. The filter paper with the insoluble proteid residue was placed in a Kjeldahl flask and the nitrogen in it determined in the usual way.

Salt-soluble nitrogen.—After removing the water-soluble proteids the flour residue was placed in a flask and 500 cc of 5 per cent sodium chlorid solution added. After standing for about two hours at 30° the nitrogen in the extract was determined as in the case of proteid soluble in water.

Gliadin.—Five grams of flour or other material were weighed into a flask and 250 cc of 70 per cent alcohol added, the contents of the flask being shaken at intervals of half an hour for three hours. The alcohol was left in contact with the flour for about eighteen hours. The alcoholic extract was then filtered and 100 cc of the filtrate placed in a Kjeldahl digestion flask connected with a condenser. The alcohol was removed by distilling and the nitrogen in the residue determined in the usual way. With old or unsound flours a correction must be made for soluble amid bodies.

Glutenin.—The residue from the gliadin determination was washed with 70 per cent alcohol until the washings no longer gave a reaction for proteids. It was then transferred to a flask and 200 cc of a 5 per cent solution of sodium chlorid added to remove globulin and other proteids. After two hours' extraction the residue was washed with distilled water and transferred to a flask, 250 cc of a 0.2 per cent solution of potassium hydroxid added, and after three hours' extraction the solution filtered, and the nitrogen in 200 cc of the filtrate determined in the usual way. The glutenin determination is the most troublesome of all, because the other proteids must be removed before it can be extracted with potassium hydroxid. For all practical purposes the glutenin may be estimated by difference. Results thus obtained are within 0.1 per cent of the actual amount recovered with potassium hydroxid.

Amid nitrogen.—Fifteen grams of flour were treated as described for the determination of water-soluble proteids. Fifteen cubic centimeters of Stutzer's copper solution were added to 200 cc of the filtrate and the albuminoid nitrogen determination was made in the usual way. The difference between the nitrogen of the water-soluble proteid and the albuminoid nitrogen represents the amid nitrogen.

Tannic acid was also used to precipitate the total soluble proteid matter, and gave satisfactory results. The usual method for the determination of albuminoid nitrogen is not applicable to flour because a paste-like mass is formed which can not be readily filtered.

In following out this method in analyses made in our own laboratory we had no difficulty in filtering off the water extract from leguminous grains, but we found it practically impossible with flour. However, I think these separations could be made by means of centrifugal force. We have not been able to make a thorough test of this method, so have not thought it well to give any of the results obtained.

The PRESIDENT. The report of the associate referee on meat proteids is now in order.

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[a] Minn. Exp. Sta. Bul. No. 63, 1899.

REPORT ON SEPARATION OF MEAT PROTEIDS.

By W. D. Bigelow, *Associate referee.*

This subject has been again divided into the nitrogenous constituents of meats, and the nitrogenous constituents of meat extracts. The associate referee has been fortunate in securing the collaboration of Mr. Grindley, who has devoted so much attention to the study of meats and includes in his work a careful comparison of the amount of proteids precipitated by various reagents in the water extracts of meats, both raw and cooked. Mr. Grindley's report is submitted as a separate paper.

During the last year the study of the separation of the proteids in meat extracts, which has been conducted in the food laboratory of the Bureau of Chemistry, was continued by W. D. Bigelow and F. C. Cook for the purpose of confirming previous results, and of studying the action of combinations of reagents which had not previously been tried in that laboratory. Before reporting on the work of the past year it has been thought best to give here, in greater detail than has already been published, the results of previous work by the referee and R. Harcourt.

Considerable attention has been given in recent years to the method for the precipitation of proteids by means of bromin, as suggested by Allen and Searle,[a] and modifications of that method which have since been published. A careful study was made of this method with a view to determining the conditions under which it would yield the maximum results. After repeated trials it was decided that the filtration could not be successfully performed under less than two hours after precipitation. We were unable to filter the mixture under one-half hour, as directed by Allen and Searle, owing to the incomplete separation of the precipitate. Aliquot parts of the filtrates from the proteids coagulated by heating were subjected to the action of bromin water in some cases, and in others saturated with bromin. In all cases the solutions were acidified with hydrochloric acid, as directed by Allen and Searle, before the addition of the bromin water or bromin.

Table I.—*Bromin precipitate in filtrate from coagulated proteids.*

Description of sample.	Precipitate by bromin water filtered after 2 hours.	Precipitate by saturation with bromin filtered after 16–18 hours.
	Per cent.	*Per cent.*
Hammond's beef extract	1.32	1.31
A. & J.'s liquid peptones	.04	.04
Tropon	.05	.06
Arlington Chemical Co.'s beef peptones	.22	.34
Arlington Chemical Co.'s liquid peptonoids	.03	.09
The London Essence Co.'s essence of chicken	1.32	1.26
Rose's peptonized beef	2.40	2.52
Extractum carnis Liebig	.99	.86

It would appear from the figures given above that the results by allowing the liquid to stand over night before filtration are practically identical with those obtained by filtering within two hours after precipitation. The former method gives filtrates that are more uniformly clear, and it can be used with greater economy of time. It was therefore chosen for subsequent work in comparison with other methods. The

[a] Analyst, 1897, **22**: 259.

details employed were those published in Bulletin 65 of the Bureau of Chemistry, page 11.

Phosphotungstic acid was suggested by Stutzer. It has long been conceded that all proteids except peptones were precipitated by ammonium sulphate.[a] Bömer[b] substitutes zinc sulphate for ammonium sulphate. He acidifies the filtrate from the insoluble and coagulable material with 1 cc of 1—4 sulphuric acid to prevent the precipitation of zinc phosphate, and saturates the solution with zinc sulphate. The precipitate is washed with a saturated solution of zinc sulphate and its nitrogen content determined by the Kjeldahl method. It is our purpose here to record the results of a careful study of the foregoing methods.

For the purpose of comparison Mallet's method was employed in the work reported herewith. In some cases a preliminary addition of tannin was made, and in others the tannin was omitted and the phosphotungstic acid added directly to the filtrate from the coagulated proteids. It is regretted that Schjerning's method, published while the work here reported was in progress, in which tannin and sodium chlorid are used as precipitants, did not come to our attention in time to be included.

The action of flesh bases of the reagents employed in the various methods was also studied. Samples of glycocin, leucin, alanin, asparogin, glutamin, tyrosin, betain, kreatin, kreatinin, hypoxanthin (sarkin), carnin, and allantoin, were obtained in as high a state of purity as practicable, dissolved in water acidified with hydrochloric acid and successive small portions of the solution treated with bromin, tannin, phosphotungstic acid, ammonium sulphate, and zinc sulphate. No precipitation occurred in any case with bromin, ammonium sulphate, or zinc sulphate. Tannin gave a very slight turbidity with alanin and betain.

On boiling the solution and allowing it to cool, this insoluble matter subsided and was separated by filtration. Further concentration of the filtrate did not cause a reappearance of this turbidity, which was evidently due to a slight trace of an impurity which had not been removed from the preparations employed.

Phosphotungstic acid either produced no precipitate or a precipitate which dissolved on heating. When the solutions were heated to from 85° to 90° no insoluble matter was left in any case, except that alanin and betain retained a scarcely perceptible turbidity, as they did with tannin. As was stated, under tannin this was doubtless due to a trace of an impurity in the preparations employed. These tests are in part a repetition of Mallet's work, but it is deemed appropriate to mention them here in confirmation of his results.

The reagents mentioned were then employed for the examination of a number of samples of meat extracts and similar products, the results of which are shown in Table II. The percentage of material insoluble in water, of material coagulated by boiling after slightly acidifying, were first determined by the methods outlined in Bulletin 65 of the Bureau of Chemistry. Aliquot parts of the filtrate from the proteids coagulated by boiling were then treated with the reagents mentioned above. It will be observed that no relation exists between the proteids precipitated by bromin and those precipitated by zinc sulphate.

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[a] Stutzer, Zeit. anal. Chem., 1895, 5: 372.

[b] Zeit. anal. Chem., 1895, 5: 562; Bureau of Chemistry Bul. No. 65, p. 18.

TABLE II.—*Nitrogenous bodies in meat extracts (Bigelow and Harcourt).*

[Expressed in terms of total nitrogen in original sample.]

Serial No.	Substance.	Total.	Insoluble in water.	Precipitate by boiling in slightly acid solution.	Precipitate (from filtrate by boiling) by—					
					Bromin.	Zinc sulphate.	Bromin filtrate in zinc sulphate.	Zinc sulphate + bromin.	Phosphotungstic acid.	Bromin and phosphotungstic acid.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
18239	Beef extract, paste	8.43	.....	.....	13.88	6.168	.....	.....	63.82	58.36
18631	Liquid food	1.90	14.74	79.48	.00	.00	.00	.00	1.579	1.579
18633	Meat powder, digested	12.13	.495	.....	83.51	82.00	4.1	86.1	91.92	66.78
19398	Beef extract, paste	8.88	2.252	.34	14.75	12.95	3.378	16.44	.....	23.65
19399	Beef extract, fluid	3.04	.....	.....	3.29	1.65	8.22	9.86	10.53	22.36
19456	Meat powder, digested	14.41	94.59	.14	.42	.42	.....	.....	.....	.56
19457	Liquid peptone	.25	.00	8.00	12.00	8.00	8.00	16.00	.....	24.00
19458	Beef peptonoids, powder	4.27	68.62	10.77	7.96	8.43	2.81	11.24	.....	10.54
19459	Liquid peptonoids	.34	.00	.00	26.47	8.82	17.65	26.47	.....	32.55
19460	Meat powder	12.03	57.19	.17	15.71	8.90	.....	.....	23.69	22.69
19461	Commercial peptone, fluid	.95	.00	.00	34.74	18.95	.....	.....	52.63	44.21
19462	Beef tonic, fluid	.19	.00	.00	21.05	68.42	.....	.....	42.10	42.10
19463	Beef extract, powder	2.05	50.24	7.81	8.29	40.00	.....	.....	15.61	14.63
19464	Essence of chicken, fluid	3.43	.....	2.04	36.71	27.39	33.51	60.90	69.64	.....
19465	Fluid beef, paste	7.15	11.05	.00	29.79	40.98	11.75	52.73	59.86	62.10
19466	Beef extract, paste	3.23	50.16	.00	21.05	21.36	.....	.....	17.64	28.48
19467	Peptonized beef, gelatinous	6.27	11.48	2.39	27.91	36.52	.....	.....	74.32	85.96
19766	Beef juice pressed at 60°.	1.09	20.18	24.77	11.01	38.53	.....	.....	14.68	21.10
19767	.....do	1.18	11.02	33.90	10.17	16.93	.....	.....	8.47	15.25
19785	Beef juice pressed cold	.48	.....	70.83	4.17	6.25	.....	.....	6.25	8.33
19786	.....do	.43	.....	69.76	4.65	2.33	.....	.....	6.98	6.98
19787	Beef juice pressed after heating 6 hours	.24	.....	.....	25.00	20.83	.....	.....	50.00	41.67
19817	Liquid peptone	.31	.00	8.68	9.68	.00	.....	.....	32.26	22.58

Considering the statements of Allen and Searle, that all proteids, including peptones, were precipitated by bromin, it was intended to precipitate albuminoses by zinc sulphate, and albuminoses and peptones combined by bromin, and to calculate the percentage of peptones by the difference between the amount of albuminoses and peptones present. It was soon found, however, that not only was this difference not equal to the percentage of peptones present, but that frequently the zinc sulphate precipitate was greater in amount than the bromin precipitate. The filtrate from the zinc sulphate precipitate in the case of samples whose examination had not been completed was diluted with an equal volume of water, as directed by Allen, saturated with bromin, and allowed to stand over night, after which the precipitated nitrogenous compounds were separated by filtration, and the amount of nitrogen present determined. It was found that the nitrogen precipitated by bromin in the filtrate from the zinc sulphate precipitate was sometimes greater than was precipitated from the original solution. The sum of the nitrogen precipitated by zinc sulphate and precipitated by bromin in the filtrate from the same is usually less than that precipitated by phosphotungstic acid, although in many cases it is not materially less. It is probable that in products containing a small amount of peptones the results so obtained are sufficiently near the truth. On the other hand, in the case of commercial peptones and of meat products which have been heated for a long period, or peptonized in any manner, the use of bromin does not give reliable results even in the filtrate from the zinc-sulphate precipitate.

It is worthy of remark that, while Allen and Searle stated that all proteids were completely precipitated by bromin they only reported results obtained in the filtrate from the zinc-sulphate precipitate, and, as stated above, such results when added to the zinc sulphate precipitate are much higher than those obtained by bromin in the original solution. At the same time the nitrogen precipitated by bromin is always too low.

Since the partial publication of this work these observations on the unreliability of bromin for determining nitrogen have been confirmed in different classes of proteids by Fraps and Bizzell[a] and by Van Slyke,[b] while Schjerning[c] had previously noted that fact.

The precipitate by phosphotungstic acid was usually higher than that obtained by zinc sulphate, although in some cases it was considerably lower. The combined precipitates obtained with tannin and phosphotungstic acid did not have the effect of precipitating the peptones which were not precipitated by phosphotungstic acid, but on the other hand, gave in some cases a lower result than with the reagent alone. As stated before, this work was done before the publication of Schjerning had come to our attention, and salt was not added to the tannin reagent.

The results of this work as a whole were unsatisfactory. There appears to be no agreement between the results of different methods, and it does not seem that the products in themselves are sufficiently different in their nature to warrant the discrepancy in the results obtained. In order to obtain additional data regarding the matter, and for the purpose of comparing the tannin-salt reagent with the methods already tried, and also to study the precipitates obtained by various combinations of the reagents in use, additional work was undertaken during the last summer by W. D. Bigelow and F. C. Cook. It is felt that so far only a beginning in this work has been made.

Eight commercial products were taken of which 4 were ordinary meat products of pasty consistency; one a so-called beef juice; one a well-known digested meat powder, and two commercial peptones. A solution of each product was made, and aliquot portions were subjected to the action of bromin, zinc sulphate in saturated solution, phosphotungstic acid above 90°, and tannin-salt, respectively. The filtrate from the phosphotungstic acid reagent was allowed to cool and again filtered, and the amount of nitrogen obtained in the filtrate was added to that precipitated by the phosphotungstic acid in hot solutions, the same being given as phosphotungstic acid in the cold. The results obtained are given in Tables III and IV.

TABLE III.—*Nitrogenous bodies in meat extracts (Bigelow and Cook).*

[Expressed in terms of total nitrogen in original sample].

PRECIPITATES FROM ZINC SULPHATE FOLLOWED BY OTHER REAGENTS.

	Description of sample.	Zinc sulphate.	Zinc sulphate +bromin.	Zinc sulphate + phosphotungstic acid.	Zinc sulphate +tannin-salt.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	Meat extract	21.1	25.5	31.4	21.6
2	.....do	20.4	27.6	40.3	21.0
3	.....do	10.8	22.8	.....	11.7
4	.....do	46.1	52.6	62.5	46.5
5	Meat powder, digested	81.1	85.2	88.7	.....
6	Commercial peptone	19.9	42.2	58.9	.....
7	.....do	42.0	44.1	76.4	.....
8	Beef juice	6.2	9.9	14.3	.....

[a] J. Amer. Chem. Soc. 1900, 22: 709.
[b] U. S. Dept. of Agri., Bur. of Chem. Bul. No. 73, p. 96.
[c] Zeit. anal. Chem., 1900, 39: 545.

TABLE III.—*Nitrogenous bodies in meat extracts*—Continued.

PRECIPITATES FROM PHOSPHOTUNGSTIC ACID FOLLOWED BY OTHER REAGENTS.

Number of sample.	Phosphotungstic acid.	Phosphotungstic acid +bromin.	Phosphotungstic acid +zinc sulphate.	Phosphotungstic acid +zinc sulphate. +bromin.	Phosphotungstic acid +tannin-salt.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	41.5	44.0	42.2	44.1	41.5
2	41.7	43.3	42.5	44.6	41.7
3	40.3	42.8	41.2	43.6	40.3
4	70.4	71.4	70.8	71.5	70.4
5	93.5	93.9	98.6	104.3	95.2
6	.....	.....	.....	.....	.....
7	56.0	56.6	67.9	85.0	61.1
8	26.0	26.2	28.8	31.2	28.1

PRECIPITATES FROM TANNIN-SALT FOLLOWED BY OTHER REAGENTS.

Number of sample.	Tannin-salt.	Tannin-salt+bromin.	Tannin-salt +zinc sulphate.	Tannin-salt +zinc sulphate +bromin.	Tannin-salt +phosphotungstic acid.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	32.6	39.0	58.5	61.3	35.8
2	39.9	43.4	53.6	56.2	41.7
3	35.4	41.1	52.4	55.8	37.3
4	66.9	.....	76.0	77.5	.....
5	89.4	97.0	100.5	101.5	.....
6	53.9	62.1	76.2	77.8	.....
7	78.4	84.6	93.1	94.0	.....
8	17.0	26.3	33.1	36.3	.....

TABLE IV.—*Rearrangement of figures in Table III for purposes of comparison.*

[Nitrogenous bodies in meat extracts expressed in terms of total nitrogen in original sample.]

PRECIPITATES FROM THE FOUR REAGENTS SEPARATELY.

	Description of samples.	Zinc sulphate.	Phosphotungstic acid.		Tannin-salt.	Bromin.
			Hot.	Cold.		
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	Meat extract	21.1	41.5	42.48	32.6	23.9
2	.....do	20.4	41.7	42.22	39.9	26.2
3	.....do	10.8	40.3	40.78	35.4	14.1
4	.....do	46.1	70.4	70.63	66.9	40.2
5	Meat powder, digested	81.1	93.5	98.42	89.4	53.1
6	Commercial peptone	19.9	.....	.....	53.9	18.1
7	.....do	42.0	56.0	59.20	78.4	29.9
8	Beef juice	6.2	26.0	29.72	17.0	4.6

TABLE IV.—*Rearrangement of figures in Table III, etc.*—Continued.

PRECIPITATES FROM COMBINED REAGENTS, WITH COMPARISON OF THE ORDER IN WHICH USED.

Number of sample.	Zinc sulphate + phosphotungstic acid.	Phosphotungstic acid + zinc sulphate.	Tannin-salt + phosphotungstic acid.	Phosphotungstic acid + tannin-salt.	Zinc sulphate + tannin-salt.	Tannin-salt + zinc sulphate.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	31.4	42.2	35.8	41.5	21.6	58.5
2	40.3	42.5	41.7	41.7	21.0	53.6
3	.....	41.2	37.3	40.3	11.7	52.4
4	62.5	70.8	.....	70.4	46.5	76.0
5	88.7	98.6	.....	95.2	.....	100.5
6	58.9	.....	.....	.....	.....	76.2
7	76.4	67.9	.....	61.1	.....	93.1
8	14.3	28.8	.....	28.1	.....	33.1

PRECIPITATES FROM THE SEVERAL REAGENTS FOLLOWED (1) BY BROMIN, (2) BY ZINC SULPHATE AND BROMIN.

Number of sample.	Zinc sulphate + bromin.	Phosphotungstic acid + bromin.	Tannin-salt + bromin.	Phosphotungstic acid + zinc sulphate + bromin.	Tannin-salt + zinc sulphate + bromin.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	25.5	44.0	39.0	44.1	61.3
2	27.6	43.3	43.4	44.6	56.2
3	22.8	42.8	41.1	43.6	55.8
4	52.6	71.4	.....	71.5	77.5
5	85.2	93.9	97.0	104.3	101.5
6	42.2	.....	62.1	.....	77.8
7	44.1	56.6	84.6	85.0	94.0
8	9.9	26.2	26.3	31.2	36.3

The filtrates from the precipitates obtained by zinc sulphate, phosphotungstic acid, and tannin-salt were then divided into aliquot parts, and one part from each was treated with all four of the reagents for the purpose of determining what combination would give the highest result. It was the intention also to treat these various combinations of reagents with the ordinary simpler amids, as was done in connection with the previous work. Up to the present time, however, this has not been accomplished.

In this work the amount of nitrogen precipitated by phosphotungstic acid, even in hot solutions, was greater than that precipitated by any other single reagent, except in the case of products known to contain a relatively large amount of peptones. The precipitate obtained with phosphotungstic acid in the cold was not appreciably greater than that obtained in hot solutions. Our previous results and those of others regarding the unreliability of bromin alone are confirmed. The combined zinc sulphate and bromin precipitate are, as formerly, considerably greater than either precipitate alone. On the other hand, the sum of the phosphotungstic acid and bromin precipitates is greater in all cases than the sum of the zinc sulphate and bromin precipitates. The highest results from the combination of any two reagents were obtained by tannin-salt followed by zinc sulphate in the filtrate from the same. By adding bromin to the diluted filtrate from the zinc-sulphate precipitate from the tannin-salt precipitate an additional precipitate is obtained, which, however, is so

slight in almost all cases that it may be disregarded. The order in which the various reagents are employed has a material influence on the total amount of nitrogen precipitated by both; for instance, considerably more nitrogen is precipitated by phosphotungstic acid followed by zinc sulphate than by zinc sulphate followed by phosphotungstic acid. Again, while the highest results were obtained by the tannin-salt reagent followed by zinc sulphate, the lowest of all the combinations employed resulted from the use of zinc sulphate followed by the tannin-salt reagent.

These results are submitted merely as preliminary. They have not been confirmed, and have been obtained on so few samples that the reporter feels that no conclusion should be drawn from them. The discrepancies in the results of two reagents added in different order are surprising, and should be confirmed before being accepted. At the same time the general agreement among them suggests the advisability of continued study of the combinations of the various reagents employed.

At 12.40 the association adjourned until 1.30 p. m.

FRIDAY—AFTERNOON SESSION.

The PRESIDENT. The discussion of the subject of meat proteids will be continued by receiving Mr. Grindley's paper.

A STUDY OF THE NITROGENOUS CONSTITUENTS OF MEATS.

[A report to W. D. Bigelow, referee on food adulteration.]

By H. S. GRINDLEY.

In the following studies the fresh substance of raw and cooked meats were used entirely. In other words, the raw and cooked meats were not air-dried for analysis. In order that the character and nature of the work here reported may be more easily and thoroughly understood, the methods which were used are given in detail. It should be clearly understood that the work here reported was undertaken entirely for the purpose of research and not for commercial analysis, and therefore the prime object has been to obtain definite information as to the value and accuracy of the several methods studied, regardless of the amount of time or labor required in doing it.

PREPARATION AND ANALYSIS OF COLD-WATER EXTRACTS OF MEATS.

(a) *Preparation of cold-water extracts.*

Weigh off 9 portions of about 12.5 to 15 grams each. Transfer to beakers holding about 400 cc and add 100 cc of ammonia-free water to each lot. Stir mixture thoroughly for 30 minutes. Allow to settle for a few minutes (5–10) and filter through an 11 cm ashless and nitrogen-free filter. Collect the filtrates in 500 cc measuring flasks. See that the funnels touch the sides of the flasks so that the filtrates do not drop directly upon the liquid but flow slowly down the sides of the flasks.

Treat residues with successive portions of ammonia-free water, using about 50 cc for each extraction, stirring for 15 minutes during each extraction, and after each extraction allow the solid particles to settle for from 5 to 10 minutes before filtering. Continue this treatment until each filtrate measures about 470 cc.

Be sure each time to drain off thoroughly all the water from the residue in the beaker and also be sure to let the water upon the filter paper used in each extraction run entirely through before adding the water from another extraction. Also be careful to wash the filter paper and the residue upon the same thoroughly with each extract. These precautions are necessary in order to insure complete extraction.

Transfer carefully the extracts of about 470 cc each to a clean, dry 2-liter measuring flask, washing out each 500 cc flask twice, using about 10 cc of distilled water each time. Transfer the measuring extracts to a 5-liter flask and mix thoroughly without unnecessary agitation. Immediately measure out the volumes given below for the following determinations:

(b and c) Total solids and ash: Take 3 portions of 100 cc each.

(d) Total nitrogen: Take 3 portions of 100 cc each.

(e, f, l) Nitrogen in the form of coagulable proteids, albumoses by zinc sulphate and peptones by bromin in the filtrate from the precipitate produced by zinc sulphate: Take 3 portions of 200 cc each.

(g) Nitrogen precipitated by tannin and sodium chlorid: Take 3 portions of 200 cc each.

(h) Nitrogen precipitated by phosphotungstic acid in the cold: Take 3 portions of 200 cc each.

(i) Nitrogen in the form of ammonia: Take 3 portions of 200 cc each.

(j) Nitrogen precipitated by phosphotungstic acid in the hot: Take 3 portions of 200 cc each.

(k) Nitrogen precipitated by bromin in the cold-water extract: Take 3 portions of 200 cc each.

(l) See above (e).

(b) *Determination of total solids.*

Take 100 cc portions in triplicate. Evaporate to dryness in weighed platinum dishes upon the water bath. Clean sides and bottoms of dishes thoroughly. Heat in water oven for periods of exactly one hour until the weight is approximately constant.

(c) *Determination of ash.*

Ignite the dry residue obtained in (b) carefully over the free flame at a very low red heat until colorless or nearly so. Weigh quickly and heat again at a low red heat for only a few minutes (2 or 3) and then weigh again. Repeat this last treatment until weight is constant.

(d) *Determination of total nitrogen.*

Take 100 cc portions in triplicate and determine nitrogen very carefully as usual by the Kjeldahl method.

(e) *Nitrogen precipitated in the form of coagulated proteids in neutral solution.*

Take 200 cc portions in triplicate. Make the three portions exactly neutral to litmus paper by the addition of a N/10 solution of sodium hydrate or a N/10 solution of acetic acid. Heat the neutral solutions upon a vigorously boiling water bath stirring frequently so as not to allow the solution to foam over or to carry up the coagulated proteid upon the sides of the beaker. Continue the heating until any coagulum that forms settles completely, leaving a clear supernatant liquid. (The evaporation was usually continued until the volume of the original solution was reduced about one-half.) Filter the hot solution immediately through a well-fitting good nitrogen-free filter paper. Wash the beaker, filter, and precipitate thoroughly with boiling hot water. Determine nitrogen in the residue by the Kjeldahl method, being very careful to get off all the coagulated proteid from the beaker in which the precipitation was brought about. Evaporate the filtrate and washings to a volume of 30 cc upon the water bath and note whether or not further coagulation occurs. If coagulation occurs again filter, wash, and test as above. Save the filtrate for the following determination.

(f) *Determination of nitrogen precipitated by zinc sulphate as albumoses.*

Take the filtrates obtained from the above determinations in (e) and evaporate to a volume of 30 cc, cool to room temperature, add 1 cc of 50 per cent sulphuric acid, completely saturate with crystallized zinc sulphate. Be certain the solutions are completely saturated. About 17 grams of powdered zinc sulphate are necessary for the saturation of each 10 cc of liquid present. The saturated liquid must contain a slight excess of zinc sulphate, but much of an excess must be avoided, as it is likely to cause bumping in the subsequent determination of nitrogen. After excess of zinc sulphate has been added in the cold, warm the solution on the water bath until the solution is perfectly clear. Stir frequently while warming. Cool the mixture completely and filter through a 9-cm nitrogen-free filter paper. Wash the beaker, precipitate and filter paper thoroughly with a completely saturated solution of zinc sulphate, which is very slightly acidified with sulphuric acid (5 cc of concentrated sulphuric acid for 1,000 cc). Determine the nitrogen in the zinc sulphate precipitate by the Kjeldahl method. Save the filtrate and determine the nitrogen precipitated in the same by bromin as directed below in (l).

(g) *Determination of nitrogen precipitated by tannin and sodium chlorid.*

Take 200 cc portions in triplicate. Transfer the same to 250 cc measuring flasks, add to each 1 gram of pure sodium chlorid and a solution containing 12 per cent of tannin, until 1 drop added to the clear supernatant liquid gives no further precipitate. Dilute to the 250 cc mark, shake thoroughly, allow to stand 24 hours, filter through a dry filter, and determine the amount of nitrogen in 200 cc of each filtrate. This gives the amount of nitrogen in the form of creatin, amido-acids, and ammonium compounds not precipitated by tannin and salt. The amount of nitrogen precipitated by tannin and salt is obtained by subtracting from the total nitrogen in the aqueous extract the amount of nitrogen found in the filtrate from the tannin and salt.

The amount of nitrogen in the form of peptones is determined by difference; that is, by subtracting from the total nitrogen in the cold water solution the combined amounts of nitrogen found in (e), (f), and (g).

(h) *Determination of nitrogen precipitated by phosphotungstic acid in the cold.*

Take 200 cc portions in triplicate. Transfer the same to 250 cc measuring flasks, add to each 5 cc of 50 per cent of sulphuric acid, and shake thoroughly. To this add phosphotungstic acid (prepared as directed by Wiley) until 1 drop gives no further precipitation in the clear supernatant liquid. Dilute to the 250 cc mark and filter through a dry filter. Determine the amount of nitrogen in 200 cc of each filtrate. This gives the amount of nitrogen in the form of creatin, amido-acids, and ammonium compounds not precipitated by phosphotungstic acid. The amount of nitrogen precipitated by phosphotungstic acid is obtained by subtracting from the total nitrogen in the aqueous extract the amount of nitrogen found in the filtrate from the phosphotungstic precipitate. The amount of nitrogen in the form of peptones is determined by difference; that is, by subtracting from the total nitrogen in the cold water solution the combined sum of the amounts of nitrogen found in (e), (f), and (h).

(i) *Determination of nitrogen as ammonia.*

Take 200 cc portions in triplicate. Transfer the same to large Kjeldahl distilling flasks, add 100 cc of ammonia-free water, then about 2 grams of magnesium oxid in the form of milk of magnesia, and distill, collecting 150 to 200 cc of the distillate in 5 cc of standard hydrochloric acid. Continue the determination of nitrogen as usual.

TABLE I.—*Summary of results obtained in the determination of the nitrogenous constituents in cold-water extracts of meats.*

[Results expressed in percentages of the weights of fresh substance of meats.]

Laboratory No.	Kind of meat.	Composition of original material.															
					Nitrogen.												
							Soluble in cold water.										
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
		Water.	Fat.	Ash.	Total.	Insoluble in cold water.	Total.	Coagulated by heat.	Precipitated by zinc sulphate in filtrate from 7.	Precipitated by bromin in filtrate from zinc sulphate in filtrate from 7.	Proteid nitrogen (7+8+9).	Non-proteid nitrogen.	Precipitated by bromin directly.	Precipitated by phosphotungstic acid in hot solution.	Precipitated by tannin and salt.	Precipitated by phosphotungstic acid in cold solution.	As free ammonia.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1649	Beef, sirloin, raw	75.46	3.08	1.02	3.40	2.66	0.74	0.29	0.06	0.02	0.37	0.37	0.29	0.18	0.38	0.37	.....
1685	.....do	60.39	20.89	1.11	2.84	2.28	.56	.27	.02	.00	.29	.27	.25	.27	.26	.27	0.01
1667	Beef, neck, raw	71.29	8.77	1.02	3.10	2.47	.63	.34	.02	.01	.37	.26	.21	.29	.30	.32	.04
1671	Beef, flank, raw	59.17	24.95	.71	2.48	2.04	.44	.17	.01	.01	.19	.25	.19	.21	.23	.26	.01
1676	Beef, rump, raw	52.26	32.38	.74	2.40	1.94	.46	.22	.02	.00	.25	.21	.18	.22	.22	.23	.01
1677	Beef, round, raw	74.01	4.63	1.05	3.38	2.60	.78	.36	.03	.00	.39	.39	.25	.36	.38	.39	.02
1656	Veal, leg, raw	75.97	.96	1.15	3.47	2.62	.85	.40	.04	.01	.45	.40	.37	.39	.44	.42	.01
1662	.....do	75.53	3.99	1.10	3.23	2.58	.65	.26	.02	.02	.30	.35	.23	.24	.29	.25	.02
	Average, raw meats	68.01	12.46	.99	3.04	2.40	.64	.29	.03	.01	.33	.31	.25	.27	.31	.31	.02
1665	Beef, neck, boiled	54.40	13.68	.47	5.04	4.91	.13	.00	.02	.01	.03	.10	.00	.00	.03	.03	.01
1673	Beef, rump, boiled	38.35	42.03	.33	3.25	3.03	.21	.01	.04	.01	.06	.15	.01	.03	.07	.10	.02
1652	Veal, leg, boiled	64.73	1.59	1.01	5.31	4.88	.43	.01	.04	.02	.07	.36	.07	.06	.08	.10	.....
1653	.....do	66.65	1.31	.75	5.36	5.06	.30	.02	.05	.01	.08	.22	.03	.01	.05	.06	.00

TABLE I.—*Summary of results obtained in the determination of the nitrogenous constituents in cold-water extracts of meats*—Continued.

Laboratory No.	Kind of meat.	Composition of original material.															
					Nitrogen.												
							Soluble in cold water.										
		(1) Water.	(2) Fat.	(3) Ash.	(4) Total.	(5) Insoluble in cold water.	(6) Total.	(7) Coagulated by heat.	(8) Precipitated by zinc sulphate in filtrate from 7.	(9) Precipitated by bromin in filtrate from zincsulphate in filtrate from 7.	(10) Proteid nitrogen (7+8+9).	(11) Nonproteid nitrogen.	(12) Precipitated by bromin directly.	(13) Precipitated by phosphotungstic acid in hot solution.	(14) Precipitated by tannin and salt.	(15) Precipitated by phosphotungstic acid in cold solution.	(16) As free ammonia.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1658	Veal, leg, boiled	64.66	5.58	.59	4.80	4.61	.19	.00	.03	.02	.05	.14	.03	.02	.05	.06	.01
1664	.....do	61.87	7.77	.66	4.90	4.67	.23	.01	.04	.01	.06	.17	.03	.03	.05	.03	.02
	Average, boiled meats	58.44	11.99	.64	4.78	4.53	.25	.01	.04	.01	.06	.19	.03	.02	.05	.06	.01
1674	Beef, rump, pan broiled	27.46	47.39	1.18	3.79	3.40	.39	.01	.03	.01	.05	.34	.02	.04	.02	.02	.02
1687	Beef, sirloin, gas broiled	48.47	23.70	1.33	4.36	3.84	.52	.04	.03	.01	.08	.44	.07	.08	.08	.10	.01
1688	.....do	62.67	11.20	1.18	4.12	3.63	.48	.08	.02	.01	.11	.37	.09	.09	.09	.10	.02
1689	.....do	70.23	7.45	1.02	3.61	3.05	.56	.17	.02	.00	.19	.37	.17	.18	.19	.20	.01
1659	Veal, leg, pan broiled	65.33	5.20	1.44	4.70	4.21	.49	.01	.04	.02	.07	.43	.04	.04	.03	.01	.02
1660	Veal, leg, roasted	68.35	4.65	1.36	4.22	3.72	.50	.01	.04	.02	.07	.43	.02	.03	.04	.03	.03
	Average for last six	57.09	16.60	1.25	4.13	3.64	.49	.05	.03	.01	.09	.40	.07	.08	.07	.08	.02
	Average for all determinations	61.86	13.56	.96	3.89	3.41	.48	.13	.03	.01	.18	.30	.13	.14	.16	.17	.01

(j) *Determination of nitrogen precipitated by phosphotungstic acid in the hot.*

Take 3 portions of 200 cc each. Transfer the same to 400 cc beakers (Schott and Genossen). Add 5 cc of 50 per cent sulphuric acid, stir thoroughly, and heat the solution to boiling on the iron plate. Now add drop by drop phosphotungstic acid solution until 1 drop gives no further precipitate in the clear supernatant liquid. Maintain the boiling temperature for about 5 minutes after precipitation is complete. Immediately filter the hot solution through a nitrogen-free filter, recently prepared with the use of boiling water. While filtering keep the liquid hot (90–100° C.). Wash the beaker, filter and precipitate thoroughly with hot water, having a temperature of 95–100°. Be careful the temperature of the solution and wash water is not less than 90° C. at any time. Determine the nitrogen in the precipitate produced by the phosphotungstic acid by the Kjeldahl method.

(k) *Determination of nitrogen precipitated by bromin directly in the cold water extract.*

Take 3 portions of 200 cc each. Transfer to Kjeldahl digestion flasks, acidify with 2 cc of normal hydrochloric acid, add about 2 cc of liquid bromin, and shake the contents of the flask very vigorously for some time (2 or 3 minutes at least). If the bromin is all taken up, add more until about 0.5 cc of liquid bromin is left undissolved and the supernatant liquid is thoroughly saturated with bromin. Allow the mixture to stand for 24 hours, decant the supernatant liquid through a double quantitative, nitrogen-free filter, and wash completely the flask, residue, and filter with water thoroughly saturated with bromin. Be sure the water is completely saturated with bromin. Return the filter and precipitate to the Kjeldahl flask and determine the nitrogen as usual.

(l) *Determination of nitrogen precipitated by bromin in the filtrate from the zinc sulphate precipitate.*

To the entire zinc sulphate filtrate add 2 cc of normal hydrochloric acid, dilute with an equal volume of water, and proceed with the determination as directed above in (k).

The results obtained are tabulated below in the two tables which follow. In Table No. 1 the results are expressed in percentages of the weight of the original meat. In Table No. 2 the results are expressed in percentages of total weight of nitrogen soluble in water. The data in both these tables were taken from tables in which the results had been calculated to the fourth place decimal, and for that reason there may in certain cases be apparently slight inaccuracies in the calculation as a result of condensing the same to the form in which they are here presented.

TABLE II.—*Summary of nitrogen determinations in cold-water extracts of meats.*

[Results expressed in percentages of total nitrogen soluble in cold water.]

Laboratory No.	Kind of meat.	Nitrogen.									
		(1) Coagulated by heat.	(2) Precipitated by zinc sulphate in filtrate from 1.	(3) Precipitated by bromin in filtrate from zinc sulphate.	(4) Proteid nitrogen. (1+2+3.)	(5) Nonproteid nitrogen.	(6) Precipitated by bromin directly.	(7) Precipitated by phosphotungstic acid in hot solution.	(8) Precipitated by tannin and salt.	(9) Precipitated by phosphotungstic acid in cold solution.	(10) As free ammonia.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1649	Beef, sirloin, raw	39.30	8.00	2.60	49.90	50.10	39.37	23.82	50.73	50.17	.....
1685	.....do	48.48	2.63	.68	51.79	48.21	44.13	47.64	54.15	48.43	1.11
1667	Beef, neck, raw	54.52	3.42	.83	58.77	41.23	39.16	48.15	47.54	51.15	6.54
1671	Beef, flank, raw	39.63	2.77	1.64	44.04	55.96	43.24	48.04	51.94	57.17	2.38
1676	Beef, rump, raw	48.97	4.39	.59	53.95	46.05	40.22	48.38	48.93	49.52	2.67
1677	Beef, round, raw	46.14	3.44	.46	50.04	49.96	31.41	45.98	49.12	48.94	2.67
1656	Veal, leg, raw	46.36	5.11	1.40	52.87	47.13	43.33	45.48	51.68	49.03	1.57
1662	.....do	40.66	3.66	2.29	46.62	53.38	34.71	36.14	44.56	38.97	3.06
	Average, raw meats	45.51	4.18	1.31	50.99	49.01	39.45	42.95	49.83	49.17	2.86
1665	Beef, neck, boiled	1.58	15.42	5.60	22.60	77.40	13.53	8.16	24.34	23.05	8.99
1673	Beef, rump, boiled	4.63	19.84	4.30	28.77	71.23	4.86	13.23	33.44	48.09	8.50
1652	Veal, leg, boiled	3.22	9.06	4.13	16.41	83.59	17.23	14.71	18.08	23.37	.....
1653	.....do	5.61	16.10	4.22	25.93	74.07	8.73	2.76	15.14	20.35	1.32
1658	.....do	1.26	14.92	10.24	26.42	73.58	14.44	11.03	25.32	31.25	2.57
1664	.....do	3.13	15.55	4.01	22.63	77.37	13.00	11.37	19.91	14.63	9.43
	Average, boiled meats	3.24	15.15	5.42	23.79	76.21	11.97	10.21	22.71	26.79	6.16
1674	Beef, rump, pan broiled	2.22	7.18	1.88	11.28	88.72	6.33	10.05	.21	5.27	4.77
1687	Beef, sirloin, gas broiled	8.21	5.96	1.88	16.05	83.95	13.48	15.09	16.25	18.17	1.19
1688	.....do	17.35	4.65	1.32	23.32	76.68	19.21	18.78	18.36	19.64	3.86
1689	.....do	29.95	3.64	1.14	34.73	65.27	30.47	31.39	34.48	36.36	1.95
1659	Veal, leg, pan broiled	2.69	7.49	3.56	13.72	86.28	8.12	8.25	5.25	1.70	4.98
1660	Veal, leg, roasted	.63	8.59	4.16	13.38	86.62	5.02	6.15	8.03	5.71	6.37
	Average for last six	10.17	6.25	2.32	18.74	81.26	13.77	14.95	13.76	14.47	3.85
	Average for all determinations	22.23	8.09	2.85	33.16	66.84	23.50	24.73	30.87	32.05	3.69

DISCUSSION OF RESULTS.

In general it may be said that while the triplicate determinations upon each sample agree fairly well, the results obtained with the different methods upon different samples of meats vary greatly. For example, in No. 1671 the nitrogen precipitated by bromin forms 98 per cent of the total proteid nitrogen, while in No. 1677 the nitrogen precipitated by bromin forms only about 63 per cent of the total proteid nitrogen. A comparison of the other methods show similar results. It does not seem probable that different cuts of meats or that meats from different animals would be so different in composition as to give such results. On the other hand, the several determinations have been made with the greatest precautions, and it does not

seem possible at the present writing that such large variations can be due to the methods as now used. Further investigation of this subject is now under way, and it is hoped that the additional results which will soon be obtained will lead to a solution of this question.

(1) *Nitrogen coagulated by heat.*

In connection with the investigations which are being made in this laboratory it seems quite desirable to determine directly the amount of coaguable proteids contained in water extracts of meats. At first much difficulty was encountered in this determination in getting even fairly concordant results. In our work even a very slight excess of acetic acid has decidedly interfered with the complete separation and proper coagulation of the coaguable proteids. Under such conditions the proteid separates as a slimy film upon the surface of the liquid, the coagulation is not complete, and filtration takes place very slowly. In all such cases an additional separation takes place upon further evaporation of the filtrate for the precipitation of albumoses. In fact, separation of additional proteid continues until the solution is evaporated to dryness upon the water bath. This incomplete coagulation resulted even when 2 cc of N/10 acetic acid was used. Such difficulties were not so apparent in the analysis of cold-water extracts from cooked meats, which, however, contained as a rule very slight amounts of coaguable proteids.

In order to overcome these difficulties the coagulation in the later determinations was brought about in a neutral solution, after some preliminary experiments had proved conclusively that under such conditions the precipitation of coaguable matter was more complete, that the filtration under such conditions always took place readily, and that more concordant results were obtained. It was also proved that the coagulation was more complete when the solutions were made neutral to litmus paper than it was when the solutions were made neutral to phenolphthalein. This fact is shown by the following data:

TABLE III.—*Coagulation in neutral solutions.*

Solutions.	I.	II.
	Per cent.	*Per cent.*
Total nitrogen in water extract	0.765	0.755
Nitrogen coagulated in solution neutral to litmus paper	.303	.303
Nitrogen coagulated in solution neutral to phenolphthalein	.266	.269

In order to make the solutions from the water extracts of meats neutral to phenolphthalein it in all cases required the addition of 1 to 3 cc of N/10 sodium hydroxid more than it did to make the solutions neutral to litmus paper. Further studies are now being made upon this subject.

(2) *Nitrogen precipitated by bromin.*

The results reported in this connection confirm Bigelow and Harcourt's statement,[a] namely, "that the bromin precipitation from the original solution was found to hold only a small and variable portion of the proteids present." W. D. Bigelow,[b] also says "that more recent results in this laboratory indicate that bromin does not precipitate from aqueous solution all the proteoses and peptone present; at the same time, considering the small amount of these bodies contained in meat, it is believed that the results are approximately correct."

Van Slyke[c] and also Schjerning[d] have shown that bromin does not completely precipitate milk proteids and their derived caseoses and peptones.

[a] J. Amer. Chem. Soc., 1901, **23**: 8, Proceedings.

[b] U. S. Dept. Agr., Bureau of Chemistry, Bul. No. 13 (part 10), p. 1396.

[c] Chem. News, 1903, **88**: 92.

[d] Zeit. anal. Chem., 1900, **39**: 545.

Fraps and Bizzell[a] concluded from a study of the application of the bromin method to the determination of vegetable proteids that bromin is not a suitable precipitant for proteids in vegetable materials.

It is evident from the results here presented that bromin does not even precipitate all of the proteid which is coagulated by heat in the case of raw meats. Assuming that the sum of the nitrogen coagulated by heat, precipitated by zinc sulphate, and precipitated by bromin in the filtrate from the zinc sulphate precipitate represents the total proteid nitrogen in the water extracts of meats, then the bromin precipitates 77.37 per cent of the proteid nitrogen in raw meats, 50.31 of the proteid nitrogen in boiled meats, and 73.48 per cent of the proteid nitrogen in broiled or roasted meats which is soluble in water.

(3) *Nitrogen precipitated by phosphotungstic acid in hot solution.*

The results obtained in this work show that phosphotungstic acid at a temperature between 90° to 98°, fails to precipitate all of the proteid material. In fact, it gives but slightly higher results than does bromin.

Mallet[b] proposed the use of phosphotungstic acid at 90° for precipitating proteids.

Fraps and Bizzell[c] found in their work that phosphotungstic acid did not precipitate vegetable proteids completely at 90° or 100°, but that this reagent at 60° precipitates very nearly the same quantity of nitrogen (with vegetable materials) as copper hydroxid.

(4) *Nitrogen precipitated by tannin and salt and nitrogen precipitated by phosphotungstic acid in the cold.*

The amount of nitrogen precipitated by these reagents in a number of cases are nearly alike, in other cases there is a wide difference in their precipitating power. The average results, however, show that in raw, broiled, and roasted meats they precipitate about equal amounts of nitrogenous substances, while in the water extracts of boiled meats, the phosphotungstic acid precipitates more than does the tannin and salt.

(5) *Nitrogen as free ammonia.*

The results show that there is a small amount of nitrogen as ammonia or ammonium salts.

The PRESIDENT. I believe there is also a paper on the separation of proteids in cereals to be presented.

DETERMINATION OF GLIADIN AND GLUTENIN IN FLOUR BY THE FLEURENT-MANGET METHOD.

By JOSEPH S. CHAMBERLAIN.

MOISTURE.

Moisture was determined in the air dry sample by the following method suggested by A. McGill, referee on cereal products:

It is impractical, if not impossible, to dry flour and similar starchy material to constant weight below a temperature of 105° C. If exposed to air at this temperature for any considerable time, a notable increase of weight by oxidation occurs, especially in the presence of fats. For this reason an atmosphere of hydrogen or coal gas is necessary, and especially is this true if any component is to be estimated by difference, when the error in water determination enters into such estimation. The error due to the cause named may easily reach 2 or 3 per cent.

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[a] J. Amer. Chem. Soc., 1900, **22**: 709.

[b] U. S. Dept. of Agr., Div. of Chem., Bul. No. 54.

[c] J. Amer. Chem. Soc., 1900, **22**: 709.

On account of the convenience of coal gas this is generally used; and a very practical form of drying oven can be made by inserting a metal tube of 4-inch diameter through the oil bath used in hydrolysis of starch. This tube may have ends clamped on gas-tight and carrying nipples fitted with rubber tubing by which the dry gas is conveyed from the supply pipe. A tin tray to fit the bottom of the tube makes it easy to manipulate the samples to be dried; and these must be weighed in ordinary weighing bottles, or paired watch glasses, owing to the great hygroscopicity of the dry starch. Two hours' exposure at 105° C, using 2 grams of material and about 2 cubic feet of carefully dried coal gas per hour is sufficient for perfect desiccation.

Increase of weight through oxidation by air is very slow, indeed negligible for most purposes at temperature below 80°, so that the preliminary drying of the samples at this temperature may be effected in air, if desired.

Owing to the great hygroscopicity of dry starch, it is not recommended to weigh out from the dry sample. It is preferable to let the sample come into hygroscopic equilibrium with the air, and to keep it in carefully closed containers.

The results obtained by this method on the three samples of flour were as follows:

Serial No.	Moisture.
	Per cent.
6534.....	13.79
6535.....	12.25
6536.....	12.77

All of the results in this report are determined on air-dry material and calculated to dry material on the basis of the above results for moisture.

The Fleurent-Manget Method.

Fleurent[a] gives the following method for the determination of gliadin and glutenin in flour:

A potash solution containing from 3 to 3.5 grams of potassium hydroxid per liter is prepared with 70 per cent alcohol. The gluten from 33.4 grams flour is triturated in a mortar with portions of the alkaline solution, and the liquid transferred to a stoppered flask until 150 cc is made up. In the flask, to which some glass beads are added, the liquid is violently shaken during an hour until the gliadin is completely dissolved and the glutenin emulsified. On passing carbon dioxid into the liquid, the glutenin is precipitated, and the clear liquid, containing the gliadin, is (in aliquot part) dried at 105°, after filtration, and weighed. From this weight must be taken the weight of carbonate of potash in 50 cc of the solvent; the difference is the weight of dry gliadin, and can easily be calculated to a percentage on the flour. The glutenin per cent is determined by subtracting the gliadin from the total gluten, determined on a separate portion of the flour.

From extensive acquaintance with wheat flours of commerce, Fleurent gives the following limits: Glutenin, from 18 to 40 per cent gluten; gliadin, from 32 to 60 per cent gluten; and adds:

Bread made with a flour whose gluten contains as little as 20 per cent glutenin rises well during fermentation, but flattens in the baking. One always uses too much water with such a flour. When the gluten approaches 34 per cent of glutenin, the dough neither rises during fermentation nor in the oven, and the bread remains heavy and indigestible. Variations of 2 per cent in glutenin (from the normal 25 per cent) give rise to differences in the bread which are quite recognizable to the expert baker.[a]

Dr. Ch. Manget[b] suggests the following improvements upon Fleurent's process:

Alcohol of 71 per cent is employed because the water in the gluten reduces the strength to 70 per cent.

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[a] Manuel l'analyse chimique, 1898, pp. 308–314.

[b] Rev. intern. des falsif., 1902, p. 91.

The alcoholic potash solution is made as follows: Alcohol of 95 per cent at 15°, 775 cc; aqueous potassium hydroxid of 22 grams, per liter, 100 cc; distilled water, to make 1 liter.

In order to obtain a correction for the weight to be deducted from the gluten extract a mixture of 100 cc of the alcoholic potash with 50 cc alcohol (of 71 per cent) is saturated with carbon dioxid and one-third of the resulting solution evaporated to dryness at 105°. (Weight=R).

The determination is made by washing out the gluten from 30 grams of flour with a solution of chlorid of sodium (1 gram per liter). The separated gluten is air dried for one hour and weighed (moist gluten).

It is then shaken up with 100 cc of the alcoholic potash, in a stoppered bottle, furnished with glass beads, until a uniform emulsion is formed. This is transferred to a flask marked at 150 cc (or, better, specially graduated to allow for the volume of the moist gluten) and the washings of the bottle (71 per cent alcohol) are added, to make up to the mark. After very thorough agitation, 50 cc is poured out and dried, after supersaturation with carbon dioxid at 105°. The residue, less "R," gives dry gluten. The difference between this number and that previously found, gives moisture in moist gluten. The remainder of the solution is treated with carbon dioxid in excess, and 50 cc is filtered off and dried at 105°. The residue is gliadin from 10 grams flour (together with the weight R, which must be deducted). Glutenin is obtained by difference.

In the following work this method was followed, with two exceptions: (1) A 1 per cent sodium chlorid solution was used as wash water for determining gluten instead of a 0.1 per cent solution. This modification was introduced because it had been found from previous work that the more dilute the salt solution, the more gliadin was dissolved in washing and lost. (2) In obtaining the glutenin by subtraction Fleurent's original method was used and the gliadin subtracted from the weight of dry gluten dried at 105° to 110° instead of from the dry gluten as determined by Manget. At the same time that aliquot portions of the gliadin-containing filtrate were taken for the determination just described, similar portions were taken for nitrogen determinations, which were made by the Gunning method by T. C. Trescot, of the Bureau of Chemistry. The results obtained for gliadin and nitrogen are given in Table I:

TABLE I.—*Comparison of gliadin by weight and by nitrogen determinations.*

[On dry flours.]

Sample number.	Description of sample.	Gliadin.			Nitrogen in gliadin.
		By Fleurent's method (weight).	By nitrogen determination.		
			N×5.68.	N×6.25.	
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
6534	"Straight" flour from Kansas hard winter wheat.	8.77	7.78	8.56	16.43
		8.60	7.52	8.27	
6535	"Straight" flour from imported macaroni wheat.	12.72	11.42	12.56	16.18
		12.44	11.30	12.25	
6536	"Straight" flour from North Dakota macaroni wheat	11.84	10.68	11.75	15.97
		11.83	10.56	11.62	
		11.95	.....	.....	
		11.97	.....	.....	

It will be seen that the results from the nitrogen determinations agree quite closely with those obtained gravimetrically if the factor 6.25 is used. This fact is apparently substantiated by the determination of the percentage of nitrogen in the dry gliadin determined by Fleurent's method. The gliadin residues were washed into a Kjeldahl flask and the nitrogen determined with the results given in the last column of Table 1. These results agree quite closely with the theoretical 16 per cent of nitrogen corresponding to the proteid factor 6.25, but do not agree with the per cent 17.60

corresponding to the factor 5.68. It must be said, however, that there is no evidence that the weight of gliadin taken, upon which the per cents are figured, represents pure proteid. On the contrary, the probabilities are that the residue, after allowing for the potassium carbonate formed in the neutralization of the alcoholic potash solution, does not consist of perfectly pure gliadin.

Osborne and Voorhees [a] give 17.66 per cent of nitrogen in gliadin and 17.49 per cent in glutenin, purified as perfectly as possible, and use 5.68 as the factor for the two corresponding to a nitrogen content of 17.60 per cent. These results, if accepted as correct, show that the gliadin as determined by Fleurent's method is not pure proteid, although it is more nearly so than the gluten itself, as is shown by succeeding analyses. That this is so, and also that the gliadin as obtained by Fleurent's method is not the gliadin as it exists in the wheat, is further indicated by the following results.

By the method as described, as well as in many analyses of wheat and flour made by other methods, the gliadin and glutenin are determined in the gluten as obtained by washing out in water or dilute salt solutions. The following facts, bearing upon the question of the composition of gluten and its relation to the proteids of flour, were obtained by washing out the gluten in a slow stream of a 1 per cent sodium chlorid solution and then determining the percentage of nitrogen in the dry gluten. These results are given in Table II.

TABLE II.—*The composition of gluten in relation to the proteids of flour, with special reference to the effect of the 1 per cent sodium chlorid washing solution.*

[Calculated to dry flour.]

Sample number.	(1) Gluten. (By washing in 1 per cent NaCl solution.)	(2) Nitrogen in gluten.	(3) True gluten. (N. in gluten ×5.68.)	(4) Nonproteid in gluten. (1−3.)	(5) Total proteid in flour. (N×5.68.)	(6) Proteid lost in washing. (5−3.)	(7) Proteid soluble in 1 per cent NaCl solution.	(8) True proteid. (3+7.)
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
6534	15.10	13.63	11.64	3.46	14.43	2.79	3.07	14.71
	14.61	13.24	10.96	3.65	14.37	3.41	3.29	14.25
6535	18.40	12.80	13.40	5.00	17.83	4.43	4.09	17.49
	17.60		12.78	4.82	17.72	4.94	4.43	17.21
6536	15.48	13.60	11.93	3.55	16.07	4.14	4.15	16.08
	15.37	13.59	11.87	3.50	16.06	4.19	3.69	15.56

On the glutens as given in column 1 the percentage of nitrogen was determined by the Gunning method, with the results given in column 2. These figures show that dry gluten, as ordinarily obtained and weighed, and on which the results in Fleurent's method are based, contains several per cent of nonproteid material. From the nitrogen determinations are obtained the amounts of proteid actually present in the gluten which are given as per cents of dry flour in column 3 of the above table. By subtracting these results from the gluten as weighed the amount of nonproteid material present in the gluten is obtained as given in the following column. These figures represent the material present with the gluten and considered as such, which should not be included, as it is not proteid. Consequently if the results for gliadin and glutenin are determined on this basis they can not be correct.

Again, from the true gluten determination, it is seen that not only does the gluten as washed out contain nonproteid material but the amount of proteid found in the gluten is less by several per cent than the total proteid contents of the flours. The difference between the total proteids found by nitrogen determination and the true gluten, or the amount of real proteid present in the gluten, is shown in column 6 of

[a] Amer. Chem. J., **15**: 469.

Table II, and indicates that there is a loss of from 2.79 to 4.94 per cent of proteids in the washing out of the gluten with a 1 per cent sodium chlorid solution. This result was to be expected, as a certain amount of the proteids of flour is known to be readily soluble in dilute salt solutions. In column 7 is given the percentage of the proteids found to be soluble by extraction in a 1 per cent sodium chlorid solution, and these figures agree in general with those given in the preceding column. If to the true gluten is added the amount of proteid soluble in the washing solution the total proteid present in the flour is obtained. These figures, as given in the last column of the table, should agree in general with the results for total proteids based on nitrogen determinations as given in column 5.

The determination of gliadin and glutenin, therefore, by Fleurent's method, using the gluten obtained by washing out with a 1 per cent sodium chlorid solution, does not give accurate results unless corrections are made both for the nonproteid material contained in the gluten and for the loss in washing of all, or nearly all, of the proteids soluble in a 1 per cent sodium chlorid solution. It remains to show whether or not the soluble proteid obtained from the gluten by solution in alcoholic potash and the subsequent precipitation of the insoluble proteid by neutralization with carbon dioxid is in reality gliadin, or whether with the gliadin other proteids are included.

Extraction of Flour with Salt Solutions and with 70 per cent Alcohol.

As previously stated it was found that the more dilute the salt solution used for extraction the more gliadin or alcohol-soluble proteid was dissolved. By extracting the air-dry flour with salt solutions of varying strengths the following results were obtained:

Table III.—*Average amounts of proteid extracted by salt solutions of varying strength.*

[Calculated to dry flour.]

Sample number.	Sodium chlorid.			Potassium sulphate, 5 per cent solution.
	1 per cent solution.	5 per cent solution.	10 per cent solution.	
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
6534	3.29	2.73	2.20	2.16
6535	4.43	3.46	3.36	3.29
6536	4.15	3.52	2.83	2.73

It is thus shown that a 5 per cent solution of potassium sulphate extracts practically the same amount of proteids as a 10 per cent solution of sodium chlorid. Osborne and Voorhees used 10 per cent sodium chlorid in their work, but as it was necessary to determine the nitrogen in the filtrate a 5 per cent potassium sulphate solution was adopted as preferable because it gives off no hydrochloric-acid gas during digestion. The difference between the proteids extracted with 1 per cent sodium chlorid and 5 per cent potassium sulphate represents the extra amount of proteid, probably gliadin, extracted by the 1 per cent sodium chlorid.

By extracting air-dry flour with 70 per cent alcohol (sp. gr., 0.89) for varying lengths of time and at different temperatures, it was found that extraction for from eighteen to forty-eight hours at a temperature of from 25° to 30° C. yielded the maximum amount of soluble proteid. Three extractions each for a total additional time of seventy-two hours yielded only 0.23, 0.22, and 0.20 per cent increase, respectively, of soluble proteid. It is therefore believed that a simple extraction by standing, after a thorough shaking, for eighteen to twenty-four hours, or, better, a vigorous shaking for six to eight hours, dissolves practically all of the proteids soluble in dilute alcohol. The results obtained for soluble and insoluble proteids in the 70 per cent alcohol solution are given in the first two columns of Table IV.

TABLE IV.—*Extraction with 70 per cent alcohol and 5 per cent potassium sulphate.*

[Calculated to dry flour.]

Sample number.	(1) Proteid extracted by 70 per cent alcohol (18–48 hrs.).	(2) Proteid insoluble in 70 per cent alcohol.	(3) Proteid extracted from insoluble residue by 5 per cent solution K_2SO_4.	(4) Proteid extracted directly from flour by 5 per cent solution K_2SO_4.	(5) Proteids in alcohol extract soluble in salt solution (4-3).	(6) Glutenin (2-3).	(7) Gliadin (1-5).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
6534	8.12	6.71	0.45	2.22	1.77	6.26	6.35
	8.18	6.47		2.16	1.71	6.02	6.47
6535	10.22	7.55	0.45	3.29	2.84	7.10	7.38
	10.28	7.66		3.46	3.01	7.21	7.27
6536	9.26	7.04	0.57	2.73	2.16	6.47	7.10
	9.03	7.34		2.78	2.21	6.77	6.82

It was found by extracting the insoluble residue from the alcohol extraction with 5 per cent potassium sulphate solution that only very small amounts of proteids were obtained, amounting to about 20 per cent of the total proteids soluble in that reagent. These results are given in column 3 of the above table. An average of nineteen other determinations of the same factor gave 0.60 per cent of the dry flour. This shows that the larger part of the proteids soluble in a 5 per cent potassium sulphate solution is removed when flour is extracted with 70 per cent alcohol. The residue from the alcohol extract, as given in column 2, will consist, therefore, of the glutenin plus the small amount of proteids soluble in salt solutions which has not been extracted by the alcohol. Subtracting from this insoluble residue the amount of proteids extracted from the residue by 5 per cent potassium sulphate the glutenin in the flour is obtained as given in column 6. The percentage of proteids found to be soluble in salt solution is given in column 4, while the figures in column 5 represent the amount of proteids included in the alcohol extract that are soluble in salt solution. The results given in column 7 are obtained by subtracting the figures in column 5 from the alcohol soluble proteids as given in column 1 and represent gliadin, the proteid soluble in 70 per cent alcohol, as described by Osborne and Voorhees.

DISCUSSION OF RESULTS.

In the following table the proteids as determined by the several methods and modifications are brought together for comparison with each other, and with the results given in Table IV:

TABLE V.—*Comparison of proteid determinations by various methods.*

[Calculated to dry flour.]

Sample number.	(1) Extraction method. (Sum of proteids.)	(2) By nitrogen determination. (Total proteids.) (N×5.68.)	Fleurent's method. (3) Gliadin.	Fleurent's method. (4) Glutenin.	Fleurent's method corrected. (5) True gliadin.	Fleurent's method corrected. (6) True glutenin.	Fleurent's method corrected. (7) Proteids soluble in 1 per cent sodium chlorid solution.	Fleurent's method corrected. (8) Sum of true proteids. (5+6+7.)
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
6534	14.83	14.43	8.77	6.33	7.78	3.86	3.07	14.71
	14.65	14.37	8.60	6.01	7.52	3.44	3.29	14.25
6535	17.77	17.83	12.72	5.68	11.42	1.98	4.09	17.49
	17.94	17.72	12.44	5.16	11.30	1.48	4.43	17.21
6536	16.30	16.07	11.83	3.65	10.68	1.25	4.15	16.08
	16.37	16.06	11.95	3.42	10.56	1.31	3.69	15.56

In column 1 of Table V is given the sum of the three classes of proteids named in columns 4, 6, and 7, of Table IV, determined according to the writer's method, while in column 2 is given the total proteids as calculated from the nitrogen determined by the Gunning method. In columns 3 and 4 are the gliadin and glutenin as determined by Fleurent's method, and column 5 and 6 the same figures corrected as previously described. In column 7 are given the proteid losses due to washing with a 1 per cent solution of sodium chlorid, and in the last column the sum of the true proteids obtained by Fleurent's method as corrected.

Comparing in the first place the totals obtained by the two methods as given in columns 1 and 8 with that given by the determination of nitrogen in column 2, it is seen that the sum of the proteids by Fleurent's method agrees with the latter nearly as well as does the sum by the extraction method. But comparing the amounts of gliadin and glutenin as determined by the two methods (Table V, columns 3 and 4, and Table IV, columns 6 and 7) there is seen to be a very great difference. The question at once arises—which is the true gliadin and glutenin? That the direct alcohol extract of the flour has extracted all of the alcohol-soluble proteid is shown by the preceding figures giving the amounts of proteid removed after 24 hours extraction by repeated extractions for 72 hours longer, which show that after 24 hours practically no more proteid is removed by 70 per cent alcohol. Therefore all of the gliadin must be in the alcohol-soluble extract.

That most of the proteids soluble in salt solution are also included in the alcohol extract is shown by the amounts of salt-soluble proteids left in the residue after alcohol extraction (Table IV, column 3). With this correction the alcohol extract represents all the alcohol-soluble proteid, and only that, and is therefore gliadin.

On the other hand, in the Fleurent method the gluten is first washed out from the flour, and this treatment, while it probably removes all of the proteids soluble in salt solution, also takes out a considerable portion of gliadin. More important than this is the fact that the gliadin is extracted from the gluten by dissolving or emulsifying the entire gluten with a 0.2 to 0.3 per cent alcoholic potash solution. While this is a very weak alkaline solution, still, as alkalies definitely affect the properties of proteids, it is at least questionable whether or not this treatment materially alters the solubility of these two proteids.

It seems that the Fleurent method yields too high results for gliadin and consequently too low results for glutenin. Also that the results based upon the gluten determination can not be correct because of the impure condition of the gluten. This error is not corrected by the Manget modification of Fleurent's method, as that simply gives a new procedure for determining the weight of dry gluten, without considering whether or not it is pure proteid.

Proposed Method.

In consideration of the results given above the following method, based upon the work of Osborne and Voorhees[a] and of Snyder,[b] is suggested to be used in comparison with other methods for the determination of proteids in flour:

(1) *Moisture.*—Determine moisture at 105° C. in an atmosphere of hydrogen or coal gas.

(2) *Physical condition of sample.*—Make all determinations upon air-dry flour, which should be either standard milled flour or, if laboratory prepared, capable of passing through bolting cloth 90 or 100 meshes to the inch.

(3) *Determination of proteids soluble in dilute salt solutions.*—Use a 5 per cent solution of potassium sulphate. Place 4 to 6 grams of the flour in a 150 cc or 200 cc flask and introduce exactly 100 cc of the salt solution. Shake thoroughly and allow to stand, with frequent shakings, for from 18 to 24 hours, or, better, still, agitate in a shaker for

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[a] Amer. Chem. J., **15**: 469. [b] Minn. Exp. Sta. Bul. 63, p. 519, 1899.

6 hours. After standing to settle, filter off 50 cc of the liquid and determine the nitrogen therein by the Gunning method. The amount found multiplied by 2 gives the nitrogen in the original flour. This result multiplied by 5.68 gives the amount of proteids in the flour soluble in a 5 per cent potassium sulphate solution.

(4) *Determination of alcohol-soluble proteid.*—Treat from 2 to 4 grams of flour in a similar way with 100 cc of 70 per cent alcohol, except that the extraction is made in a regular Kjeldahl digestion flask. The amount of alcohol need not, however, be exact, as the entire filtrate is used. After shaking allow to stand for from 18 to 24 hours, or, better, shake for from 6 to 8 hours. Allow the liquid to settle, filter, and wash the residue several times onto the filter paper, washing the whole thoroughly with alcohol. This is readily accomplished as the filtration is very rapid. Place the entire filtrate in a Kjeldahl flask and after strongly acidifying with sulphuric acid evaporate the filtrate until fumes appear and then determine the nitrogen by the Gunning method. Nitrogen $\times$ 5.68 equals the proteid soluble in 70 per cent alcohol.

(5) *Determination of proteids in insoluble residue from the alcohol extract.*—Return the filter paper and residue from the alcohol extract to the Kjeldahl flask and determine the nitrogen. Nitrogen $\times$ 5.68 equals the proteid insoluble in 70 per cent alcohol.

(6) *Determination of proteids extracted from the insoluble residue by 5 per cent potassium sulphate solution.*—Prepare a second alcohol extract and after returning the residue to the flask (a small flask may have been used in this case) extract as in (3) with a 5 per cent potassium sulphate solution and determine nitrogen in the extract. Nitrogen $\times$ 5.68 equals the proteids soluble in salt solution included in the insoluble residue.

(7) *Results.*—Subtract the proteids obtained in (6) from those obtained in (5) and the result is glutenin.

Subtract the difference between the proteids obtained in (3) and (6) from the proteids obtained in (4) and the result is gliadin.

Proteids obtained in (3) are the proteids soluble in salt solutions.

The PRESIDENT. If there is no discussion of the paper just read, the report on potash will be next received.

REPORT ON POTASH.

By F. B. CARPENTER, *Referee.*

In accordance with the instructions of the association at the last meeting, your reporters have confined their study to securing a method which will recover all the potash in mixed fertilizers and to the determination of moisture in potash salts.

For this work three samples were prepared, one for the determination of potash and two for the determination of moisture.

In the early part of June a circular letter was sent to about forty chemists likely to be interested in the work, asking their cooperation, and a favorable response was received from fifteen. The directions for the work were outlined in the following circular letter, which accompanied each set of samples sent to those who had expressed a willingness to cooperate:

DEAR SIR: At the last meeting of the Association of Official Agricultural Chemists the referee was directed to conduct further experiments for the purpose of securing a method that will dissolve all the potash in mixed fertilizers, giving attention to the amount of material used and the amount of water used for making the solution; also, if he has sufficient time, to study methods for the determination of moisture in potash salts. For the special study of the above subjects three samples have been prepared:

No. 1. Mixture acid phosphate and muriate of potash.
No. 2. Commercial muriate of potash.
No. 3. Commercial kainit.

DIRECTIONS FOR THE WORK.

1. Thoroughly remix material for analysis before beginning the work.

2. Moisture:

(a) Determine on all samples by official method, drying No. 1 for 5 hours in a steam bath at 100° C., and Nos. 2 and 3 in an air bath at 130° C. to constant weight.

(b) Determine on Nos. 2 and 3 by the following method: Heat 5 grams material in a platinum crucible (40 cc capacity) for 10 minutes with a small flame at a dark red heat, cool, and weigh. Repeat until constant weight is obtained.

(c) On sample No. 3 determine same as (b), covering material with about 5 grams freshly ignited lime.

3. Potash sample No. 1:

(a) Determine by official Lindo-Gladding method for fertilizers, described on pages 21 and 22, bulletin No. 46, revised edition, U. S. Department of Agriculture, Bureau of Chemistry. In making the solution, boil slowly so as to prevent any material loss of water by evaporation. In case there is considerable loss, the volume should be maintained by boiling water.

(b) Same as (a) with the addition of 20 cc concentrated hydrochloric acid before boiling.

(c) Determine in acid solution (b) by optional method described on page 22, bulletin No. 46.

(d) Determine like (a) (b) and (c) using 5 grams material instead of 10. Boil with same amount of water and make up to 500 cc as usual.

(e) Determine by any new or original method which will tend to facilitate the work or improve the results of the present official method.

4. Citrate insoluble potash, sample No. 1.

(a) Determine by method employed for citrate insoluble phosphoric acid, described on page 13, bulletin No. 46. After washing the insoluble residue from the citrate digestion, transfer to a 200 cc flask, add 20 to 25 cc concentrated hydrochloric acid, and boil until the larger part of the acid has evaporated; cool, dilute with about 125 cc water, bring to a boil, precipitate with ammonia and ammonium oxalate, cool, make up to mark, etc.; determine potash in 100 cc by the regular Lindo-Gladding method.

5. Make blank determinations of all reagents, using amounts required in regular analysis.

6. Use Gooch crucibles for filtering the potassium platinic chlorid, dissolving with hot water after weighing the precipitate and reweigh the crucibles.

7. In reporting, please describe fully any modifications of methods proposed, paying special attention to amount of material and amount of water used in making the solution.

Yours, truly,

F. B. CARPENTER, *Referee.*
M. G. DONK, *Associate Referee.*

Sample No. 1 was made up as follows:

	Per cent potash (K_2O).
Acid phosphate (23.21 pounds)	0.04
C. P. potassium chlorid (1.79 pounds)	63.00
Total (25 pounds)	4.548

Both water and acid solutions of the acid phosphate were carefully analyzed for potash, and the results were found to be the same in both cases. The percentage of potash in the C. P. potassium chlorid used in the above calculation was the average of a number of determinations made in the laboratory of the Virginia Carolina Chemical Company, together with results furnished by the associate referee.

After further work on the method for the determination of potash, it was thought desirable by your reporter, to ask those who already had samples, to test the method outlined in the following circular letter which was mailed October 31:

DEAR SIR: In connection with the A. O. A. C. potash work, I would thank you to make a determination of potash in sample No. 1 by the following modification of the Lindo-Gladding method, and let me have your result as soon as possible.

Boil 10 grams material with usual amount of water, plus 5 cc hydrochloric acid. Neutralize with sodium hydrate instead of ammonia. Otherwise follow the original method. Be careful to use only sufficient sodium hydrate to neutralize the solution.

Yours, truly,

F. B. CARPENTER, *Referee.*

Owing to the late date on which this letter was sent out, only a few responses were received. Eighteen analysts took part in the work, either in whole or in part, and 13 laboratories were represented. The following tables show the results, by various methods, as reported by individual chemists:

TABLE 1.—*Potash determinations in sample No. 1.*

(Calculated potash content, 4.548 per cent.)

Analysts.	Amount of sample.						Neutralize with sodium hydrate.		Citrate insoluble.
	Official method.		Lindo-Gladding with 20 cc hydrochloric acid.		Optional with 20 cc hydrochloric acid.				
	10 grams.	5 grams.	10 grams.	5 grams.	10 grams.	5 grams.	Water solution.	Water solution + 5 cc hydrochloric acid.	
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
F. C. Weber, U. S. Department Agriculture	4.24	.....	4.56	.....	.....	.....	.....	.....	.....
	4.22	.....	4.59	.....	.....	.....	.....	.....	.....
	4.20	.....	4.60	.....	.....	.....	.....	.....	.....
H. D. Haskins, Massachusetts station	4.29	4.37	4.51	4.47	4.22	4.28	.....	4.75	0.009
F. W. Robison, Michigan station	4.33	4.64	.....	.....	.....	.....	.....	.....	.....
	4.18	4.35	4.59	.....	.....	.....	.....	.....	.293
	4.23	4.37	4.51	4.84	.....	.....	.....	.....	.254
M. G. Donk, Florida agricultural department	4.18	4.37	4.35	4.25	.....	[a] 4.46	.....	.....	.....
	4.27	4.25	4.04	4.32	.....	4.44	.....	4.44	.....
	4.31	4.36	4.23	.....	.....	4.47	.....	.....	.....
	4.14	.....	4.44	.....	.....	4.43	.....	.....	.....
T. R. Gough, Maryland station	[b] 4.26	[c] 4.03	[b] 4.28	[c] 4.07	.....	[c] 4.13	.....	.....	.06
	[b] 4.26	[c] 4.00	[b] 4.30	[c] 4.09	.....	4.26	.....	.....	.06
H. H. Hanson, Maine station	.....	.....	.....	.....	.....	.....	.....	[d] 5.15	.....
	4.28	4.06	4.19	4.24	3.86	4.84	.....	[d] 5.88	.....
	4.29	4.44	4.20	4.06	4.44	4.48	.....	[d] 6.11	.....
W. H. Scherffius, Kentucky station	4.10	.....	.....	.....	.....	.....	.....	4.42	.....
W. G. Morrison, North Carolina station	4.49	4.49	4.58	4.54	[d] 3.58	[d] 3.36	.....	4.60	.03
C. C. McDonnell, South Carolina station	[e] 4.33	[e] 4.42	.....	.....	[f] 4.40	[f] 4.41	.....	.....	.....
G. Clausen Friend, Union Abattoir Co., Baltimore	4.49	4.55	4.66	4.70	[g] 4.55	[g] 4.59	.....	.....	.20
A. W. Bosworth, Rhode Island station	[h] 4.55	[h] 4.43	[h] 4.62	[h] 4.79	.....	[h] 4.79	.....	.....	.....
H. H. Hurt, Virginia agricultural department	[h] 4.42	[h] 4.44	[h] 4.51	[h] 4.50	[h] 4.48	[h] 4.40	.....	[h] 4.68	.08
Virginia-Carolina Chemical Co., Richmond, Va.:									
Edward Ryland	4.30	4.27	4.38	4.37	4.22	4.25	4.37	4.56	.00
S. H. Sheib	4.32	4.34	4.31	4.39	.....	.....	4.31	4.48	.....
H. M. McCance	4.22	4.18	4.26	4.38	.....	.....	.....	.....	.00
S. M. Robertson	4.31	4.26	4.31	4.34	.....	.....	.....	.....	.....
Harper Dean	4.32	4.33	4.32	4.25	.....	.....	.....	.....	.....
F. B. Carpenter	4.28	4.30	4.39	4.42	4.31	4.35	4.40	4.54	.....
Average	4.29	4.33	4.43	4.39	4.31	4.43	4.36	4.56	.10

[a] Modified as stated elsewhere.
[b] Used 5 grams to 250.
[c] Used 2½ grams to 250.
[d] Omitted from average.
[e] Final washing with 95 per cent alcohol.
[f] Water solution, final washing with 95 per cent alcohol.
[g] Used Gladding wash.
[h] No blank determinations were made.

In Table II the figures directly over the percentages represent the time of heating, expressed in hours when the sample was dried in an oven, and in minutes when ignited. The ignition was usually divided into periods of 10 minutes each, with gradual loss to last weighing.

TABLE II.—*Moistures determined in samples Nos. 1, 2, and 3.*

Analysts.	Sample No. 1.		Sample No. 2.				Sample No. 3.				
	5 hours at 100°.	Constant weight at 100°.	5 hours at 130°.	Constant weight at 130°.	Ignited 10 minutes.	Ignited over 10 minutes.	5 hours at 130°.	Constant weight at 130°.	Ignited 10 minutes.	Ignited over 10 minutes.	Ignited with lime.
	Perct.	*Perct.*	*Perct.*	*Perct.*	*Perct.*	*Perct.*	*Perct.*	*Perct.*	*Perct.*	*Perct.*	*Perct.*
F. C. Weber, U. S. Department Agriculture	..... 5.41	25 5.71	..... 2.59	30 3.24	....	40 3.88	..... 1.75	..... 2.68	....	30 9.72	..... 7.92
H. D. Haskins, Massachusetts station	6.24	.....	.....	2.58	.....	3.70	.....	1.83	.....	9.72	7.51
F. W. Robison, Michigan station	..... 6.70	24 7.60	....	....	....	....	....	....	....	....	....
M. G. Donk, Florida agricultural department	6.09	.....	.....	3.09	3.78	.....	.....	2.87	9.79	.....	8.67
T. R. Gough, Maryland station	6.12	.....	.....	2.91	.....	3.92	.....	3.82	.....	10.29	.....
H. H. Hanson, Maine station	..... 6.03	....	....	2 3.40	13 3.77	18 3.86	....	2 [a]6.84	....	21 9.34	30 7.17
W. H. Scherffius, Kentucky station	..... 5.93	$8\frac{1}{2}$ 6.02	..... 2.68	$7\frac{1}{2}$ 2.85	..... 3.71	40 3.81	..... 2.32	$9\frac{1}{2}$ 2.54	..... 9.87	60 10.31	....
W. G. Morrison, North Carolina station	6.12	.....	.....	3.30	.....	3.89	.....	2.50	.....	10.08	.....
C. C. McDonnell, South Carolina station	6.17	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
G. Clausen Friend, Union Abattoir Co., Baltimore	6.35	.....	.....	2.60	.....	3.79	.....	2.06	.....	9.39	8.28
A. W. Bosworth, Rhode Island station	6.00	.....	.....	3.22	.....	3.79	.....	2.97	.....	9.71	6.49
H. H. Hurt, Virginia agricultural department	6.22	6.30	.....	3.31	.....	3.81	.....	3.20	8.07	9.53	.....
Virginia-Carolina Chemical Co., Richmond:											
Edward Ryland	..... 6.10	....	..... 2.85	..... 3.00	..... 3.69	30 3.78	..... 2.18	..... 2.69	..... 8.56	50 9.70	..... 8.47
F. B. Carpenter	..... 6.11	....	7 2.98	12 3.24	..... 3.81	60 3.98	7 2.11	25 2.64	..... 9.78	60 10.16	..... 8.29
Average	6.10	6.41	2.78	3.04	3.75	3.84	2.09	2.71	9.21	9.81	7.85

[a] Omitted from average.

COMMENTS BY ANALYSTS.

F. C. Weber.—In moisture determinations, method (a) two results are given, and under method (c) "per cent loss from lowest weight obtained." In determinations by method (a) I was unable to obtain a constant weight after 25–30 hours, the loss in weight varying from 1 to 4 mg in sample No. 1, from 5 to 10 mg with No. 2, and from 1 to 8 mg with sample No. 3, this variation being least in the one gram samples. With method (c), sample No. 3, the material lost about 350 mg when it reached a point where it began to gain in weight, even if heated for 30 minutes and

weighed as soon as cool. The gain in weight was always proportionate to time left in desiccator. The desiccators used were newly prepared from fresh calcium chlorid.

Floyd W. Robison.—In making the solutions the flasks were connected by reflux condensers, heated to boiling, and kept boiling for exactly 30 minutes.

M. G. Donk.—Determined moisture in samples Nos. 2 and 3 by simple ignition. The percentages are based on the loss after the first 10 minutes, because after the fifth ignition the weight was not yet constant, as shown by the following figures:

Loss of weight after repeated ignitions.

Time of ignition.	Sample No. 2.	Sample No. 3.
Weight after—	*Grams.*	*Grams.*
First 10 minutes	23.6500	23.6945
Second 10 minutes	23.6490	23.6898
Third 10 minutes	23.6475	23.6858
Fourth 10 minutes	23.6457	23.6835
Fifth 10 minutes	23.6445	23.6797
Sixth 10 minutes	23.6430	.....

All possible pains were taken to heat to the same temperature each time, and at no time was a dull redness exceeded. With the muriate of potash, I can not say what causes the continued loss, but with the kainit decomposition of the salt and liberation of sulphuric acid seems to take place, as the ignited residue is alkaline.

Ignition of the kainit with lime was also unsatisfactory, for here the weight increased after the first ignition. The following are the figures of three successive ignitions of 10 minutes each:

Weight after first 10 minutes	29.0308
Weight after second 10 minutes	29.0435
Weight after third 10 minutes	29.0558

The loss after the first 10 minutes was used to calculate the per cent of moisture.

A portion of lime, ignited for several hours at a red heat, was heated in a covered platinum crucible for four successive 10-minute periods, then at a red heat for 10 minutes, next at a dull red for 3 hours, and another 3 hours at a red heat, giving the following weights in grams: 33.696, 33.846, 33.714, 33.762, 33.607, 33.703, 33.628.

It appears, therefore, that the ignition for moisture at dull redness is the temperature at which calcium oxid recombines with carbon dioxid. The variations between the figures for potash by solution with hydrochloric acid I attribute altogether to the large quantity of ammonium chlorid introduced by neutralization of the acid for precipitation, the presence of so much of this salt causing unavoidable loss by decrepitation and spattering on ignition with sulphuric acid. To overcome this I used the following modification of the optional method as used last year:

To the hot acid solution was added hot concentrated barium hydrate solution in slight excess, barely neutralized with ammonia, and precipitated lime and excess of barium with ammonium carbonate, cooled, made up to mark, and filtered. Fifty cc of the solution were evaporated to small bulk in a dish, transferred to platinum crucible, evaporated to dryness, ignited (with cover on crucible) at dull redness, dissolved residue in hot water, filtered, and the analysis completed as usual.

The use of barium hydrate introduces a minimum of ammonium chlorid, and ignition in a covered crucible avoids the possibility of mechanical loss. * * * In the sodium hydrate method the sodium introduced was not troublesome.

W. H. Scherffius.—I used a water oven for drying at 100° and an oil oven for 130°. In the latter case, one gram of material was used.

By the ignition method on sample No. 2 gradual loss was experienced for four successive ignitions of 10 minutes each, and on sample No. 3 the same loss was noted for six ignitions.

An experiment was made by drying 1 gram each of samples Nos. 2 and 3 in watch glasses in the oil oven at 120° C. The following results were obtained:

Loss in weight by drying 1 gram of sample at 120° C.

Time of heating.	Loss in weight.			
	Sample No. 2.		Sample No. 3.	
	Gram.	Per cent.	Gram.	Per cent.
5 hours	0.0259	2.59	0.0207	2.07
6½ hours	.0270	2.70	.0208	2.08
8½ hours	.0270	2.70	.....	.....

Instead of Gooch crucibles we use a form of the Fresenius filtering tube, modified by having the top expanded into the shape of a small funnel. In these tubes the precipitates can be thoroughly washed with less liquid than is required for a larger filter.

The ammonium chlorid wash in 6 or 7 successive portions of about 3 cc each is used instead of 5 or 6 of 10 cc, making the total amount used about 15 or 20 cc instead of 50 or 60 cc.

Determinations of potash on the two salts gave results as follows: No. 2, 48.88 per cent; No. 3, 12.66 per cent.

A. M. Peter.—The sodium hydrate method seems to afford a way out of the difficulty, provided the hydrochloric acid does not bring into solution more potash than is really water soluble. We must not lose sight of the fact that we are trying to determine water soluble potash, not total potash or acid soluble potash.

H. H. Hansen.—The moisture determinations on sample No. 3 were unsatisfactory either by the ignition method or drying at 130°. When heated with lime for less than 30 minutes the weight continued to decrease. When heated beyond this time the weight increased.

A. W. Bosworth.—There is an objection to the sodium-hydrate method on account of the very large amount of platinic chlorid necessary to satisfy the sodium chlorid present.

W. G. Morrison.—The optional method gives low results. It requires too many filterings, and unless the filtrates are large it is impossible to get all the potash. With an acid solution the amount of ammonium chlorid present becomes so great as to interfere with the work. The use of sodium hydroxid instead of ammonium hydroxid seems to offer no advantage, and the sodium residue with the platinum precipitate makes the process of washing much more troublesome. I prefer the official Lindo-Gladding method to any of its modifications.

Discussion of the Results.

It has been known by the association for some time that the official method gives low results on certain classes of mixed goods. Your referees have therefore endeavored to suggest changes that might tend to recover all the available potash present.

While we have not been altogether successful, it is hoped that some suggestions can be made which will be of assistance in future work.

In Table No. 1 it will be noted that the results by the official method, with one or two exceptions, are fairly concordant, both when 5 and 10 grams of material were

used, but the percentages are in most cases considerably lower than theory. The ratio of the material to the amount of water used in making the solutions seemed to have no influence on the results. The acid modification of the official method tends to give higher percentages of potash, but the results are not altogether satisfactory. The introduction of such a large amount of ammonium chlorid appears to make the method more difficult of manipulation, and while it is believed that the addition of acid is necessary to liberate all the potash, there are other sources of error which partly compensate for what has been gained in this direction. The experience of the referee has been that while there was invariably a tendency to higher results, the results were always lower than theory and less uniform than by the official method.

From later work it appears to be unnecessary to use so much acid, in which case one of the objections mentioned will be partly eliminated and the results improved. The precipitation with ammonium and ammonium oxalate, however, seems to have the effect of holding back a small percentage of the potash. Knowing that filter paper has the tendency to absorb certain salts, experiments were made to see if any excess of potash was retained by the paper when solutions were filtered; the results, however, were the same, either by filtering or decantation. The acid modification of the optional method is altogether too troublesome to be considered as a possible method in fertilizer work.

Considerable time has been spent by the referee in trying to locate the cause of the low results on potash and suggest some method by which the difficulty could be overcome.

An experiment was made on sample No. 1 as follows: 10 grams were boiled with 300 cc of water for one-half hour, neutralized with ammonia, ammonium oxalate added, and the residue filtered off and washed. This residue was again boiled with the same amount of water, filtered, and washed. An acid solution of the resulting residue showed 0.30 per cent potash. This was repeated with 5 grams of material with similar results.

From a knowledge of the composition of the sample used, it seems more probable that the potash was absorbed or occluded in the insoluble residue than that the results were due to the formation of an insoluble compound. At any rate the results on the citrate insoluble potash given in Table No. 1 indicate that practically all the potash is available for plant food. A great many modifications of the Lindo-Gladding method were tried, but there seemed to be no hope of success without the use of acid in making the solution. Before sending out the samples quite a number of determinations were made on acid solutions with some promise of improvement, but from our later work it seems that in most cases when the precipitation is made with ammonia and ammonium oxalate a small proportion of the potash is again absorbed in the precipitate.

Mr. Ryland suggested that instead of neutralizing the solution with ammonia, sodium hydrate be used. This was tried, and the results were more promising than any modification before suggested. It was thought that possibly the higher results obtained by this method were due to the admixture of soda, but several of the precipitates of potassium and platinic chlorid were dissolved and reprecipitated with no loss, showing that the additional weight was due to potash obtained from the solution. Blank determinations were made in all cases to allow for any potash that might be in the soda solution. The soda appears only to act as a substitute for the potash which is usually absorbed in the precipitate formed by the addition of ammonia and ammonium oxalate.

The principles of the method are: (1) Use a small amount of hydrochloric acid to liberate any occluded potash in the substance; (2) neutralize with soda instead of ammonia to prevent any occlusion or absorption of potash in the precipitate

usually formed by the addition of ammonia and ammonium oxalate. It is best to use only a small amount of acid in making the solution (5 cc were found to be sufficient) so as to avoid using a large amount of sodium hydrate. It is convenient to add a little phenolphthalein as indicator, thus avoiding a large excess of the alkali.

Owing to the short time which has elapsed since this modification was proposed only a few results have been obtained. These are given in Table No. 1. Some of these figures are high, probably for the reason that all of the soda was not removed from the precipitate of potassium platinic chlorid, but from the experience of the referee it is believed that this difficulty could be readily overcome in practice.

The members of the association will recall the time when the Lindo-Gladding method required the addition of 5 cc of sodium chlorid solution to the solution of potassium chlorid, just previous to the precipitation with platinic chlorid. While in this proposed method the sodium chlorid is present for another purpose, the same conditions are obtained for precipitation as in the old Lindo-Gladding method. One analyst makes objection to the method on account of the very large amount of platinic chlorid required to satisfy the sodium chlorid present. Our experience is that no larger amount of platinum solution is required than in the official method. The potash is first satisfied, and the excess precipitates a part of the sodium chlorid which is readily soluble in 80 per cent alcohol, and the remaining excess of sodium chlorid is readily removed with Gladding wash. Not enough work has been done with the method as yet to form any definite conclusions, but the results are thus far very promising.

Mr. C. L. Hare, in his report on potash for 1901, referring to the milk of lime method, says: "While the direct evaporation of the solution with platinum solution leaves a rather large amount of lime salts, none of the analysts has reported trouble in the final washings. In washing this residue analysts who have little experience with the method are apt to subject it to too vigorous rubbing. Experience teaches that excessive pulverization is unnecessary and will possibly result in loss of potash caused by finely divided potassium platinic chlorid passing through the filter." It is the latter point which should be brought out in connection with any of the Lindo-Gladding modifications which have been suggested in this report. Experiments made by the referee show that the official method has a decided tendency to lower results if the precipitate is excessively pulverized, and this may account for some of the variations in results reported in Table I.

MOISTURE.

Sample No. 1. Acid phosphate and muriate of potash.—The results by the official method, with two exceptions, are fairly concordant. The samples were carefully sealed, and such differences as occur are undoubtedly due to manipulation after opening.

Sample No. 2. Muriate of potash.—By the official method the results are not satisfactory, there being a maximum variation of 0.72 per cent, with the highest result only slightly over 3 per cent.

By the ignition method the results are more uniform, but are materially higher than those by the official method. The difference is probably due to water of crystallization in the impurities, which is only slowly liberated at 130°.

Sample No. 3. Kainit.—The referee infers that if no explanation accompanied the reports the results at 130° represent percentages obtained by drying to constant weight. Our experience has been that kainit continues to lose weight for a long time at this temperature, and this undoubtedly accounts partly for the variation in results. One analyst reports a result of 6.84 per cent by drying for two hours at 130°, which is over 3 per cent higher than any other result. The kind of oven and the manner

of inserting the thermometer undoubtedly has much to do with these variations. If this method is to be used, it would be well to specify the style of oven, and have a definite time of drying, rather than to specify "to constant weight."

The ignition method offers altogether different conditions. Here not only the mechanical moisture, but also the chemically combined water is liberated. There is also some decomposition, resulting in the loss of some of the volatile constituents. The use of lime to absorb the acids which have been liberated was not very satisfactory, as is shown by the results from different laboratories.

In using the lime method it was the experience of the referees, and several other analysts have reported the same difficulty, that after heating for a certain time the substance increases in weight. This is undoubtedly due to the absorption of carbonic acid. The method is very troublesome, and if it were not for the fact that this is practically the method used by German chemists, by which all settlements for kainit purchased from foreign producers must be made, no further consideration of the same would be recommended. It remains for this association to say whether the total water content of the salt shall be determined, or we shall adhere to the present method, with possibly some modifications, and attempt to approximate the mechanical moisture. It is possible that better results could be obtained with a somewhat lower temperature than 130°. Mr. Scherffius reports concordant results on samples Nos. 2 and 3 by drying in an oil oven for 6½ hours at 120°.

As has been suggested by several analysts, the percentage loss by the ignition method depends very much on the time of heating and the temperature employed. It is very difficult to specify conditions which will insure absolute uniformity in the hands of different analysts; the best that can be done is for each analyst to determine for himself what conditions will give him the most satisfactory results.

Recommendations.

Since the last meeting of the association nothing of especial value has been published on the determination of potash. Attention has, however, been called to the phosphomolybdic method, first elaborated by "Wavalet." The method as it stands presents no advantages over the one in present use, except that it eliminates an expensive reagent. If modified so as to be carried out volumetrically like the present volumetric phosphoric-acid method, it might prove a valuable addition to the official methods.

The results for this year do not justify any recommendation for a change in the official method for the determination of potash, but it is suggested that the modification requiring a slightly acid solution and neutralization with sodium hydrate be given further trial. The referee recommends that some action be taken to ascertain whether the members desire to determine moisture in potash salts by drying at a stated temperature or by ignition, and that the study of the method thought most desirable be continued with a view to getting more concordant results.

I wish to thank all those who have so willingly contributed their time to the work outlined by the referee and to make special mention of Mr. M. G. Donk, associate referee, and Mr. Edward Ryland, one of my assistants, who have rendered valuable assistance in the preliminary and experimental work.

The President. Is there any discussion on the points raised by the referee?

Mr. Ross. Is the theory in regard to the use of the sodium hydroxid that the energies of the precipitate are directed toward an occlusion of the sodium salts rather than the potash salts?

Mr. CARPENTER. That is the only explanation I can offer.

Mr. ROBINSON. Would it not be possible to use asbestos to carry down the precipitate mechanically, and keep it from creeping?

Mr. CARPENTER. I do not see that there would be any objection if we knew that the asbestos had no potash in it.

Mr. CAMERON. There is this objection: The majority of the samples of asbestos on the market are magnesia bearing, and the magnesia would probably precipitate much more of the platinum reagent.

Mr. WILLIAMS. Some time ago I made an analysis of asbestos which contained over 3 per cent of total iron. I did not determine whether it was ferrous or ferric.

Mr. HUSTON. In making determinations of moisture in my laboratory on mixtures of phosphates and potash salts it was found that fumes of hydrochloric acid were continually given off from a mixture of hydrochloric acid and muriate of potash, and accurate moisture determinations were exceedingly difficult to obtain. I presume the same difficulties would occur in dealing with sample No. 1. In fact, after working on the samples a while the copper of the baths was strongly attacked by the hydrochloric acid which was given off by the mixture of acid phosphate and only a small amount of muriate of potash, certainly less than was contained in sample No. 1.

The PRESIDENT. If there is no further discussion the report of the committee on nominations will be received.

REPORT OF COMMITTEE ON NOMINATIONS.

Mr. WHEELER. On behalf of the committee I place the following names in nomination: For president, Mr. M. E. Jaffa, of California; for vice-president, Mr. C. L. Penny, of Delaware; for secretary, Mr. H. W. Wiley, of Washington, D. C.; as additional members of the executive committee, Mr. W. P. Headden, of Colorado, and Mr. W. R. Perkins, of Mississippi.

Mr. VAN SLYKE. I move that the nominations be approved and the officers named be declared elected accordingly.

The motion was unanimously carried.

The PRESIDENT. The report on soils is now in order.

REPORT ON SOILS.

By F. P. VEITCH, *Referee.*

Only two laboratories have participated in the work on soils. Mr. Seidell, of the Bureau of Soils, Department of Agriculture, made an examination of methods for the analyses of alkali soils; Mr. Moore and the referee worked on methods for determining available plant food, while the referee alone devoted a great deal of time to the study of acidity and methods for its determination. The cooperative work for 1903 was outlined in the following circular:

Outline for A. O. A. C. Work on Soils for 1903.

Owing to the fact that we have no methods which may be regarded as furnishing an accurate measure of the available plant food of a soil, it does not seem advisable to the referee to ask chemists to compare their results on a particular set of soil samples. For this and other reasons, not necessary to state here, it appears more rational to request those who can take part in the soil work to secure their samples from soils of which they know the crop history and fertilizer requirements, and endeavor to determine the available plant food in these samples by the methods mentioned below or by any other methods deemed worthy of trial. As the number of samples worked on by each chemist will be small, several methods may be tried without overburdening the individual. The results, together with the crop and fertilizer history of the soils, will be summarized by the referee and it is hoped to get comparisons of several methods on a large number of samples of known soils. It is particularly requested that those chemists dealing with soils which have never shown the need of fertilizers will cooperate heartily in the work in order that we may at least begin to establish standards with which to compare fertilized soils.

The lines of work upon which cooperation is requested are the following:

(1) Determination of available potash and phosphoric acid of virgin and cultivated soils in samples taken from each well-marked stratum of soil to the depth of four feet.

(2) Investigation of the most desirable amounts of soil and water to be used in dissolving the soluble salts of alkali soils, and of the time required for the establishment of equilibrium between the soil and the solvent.

(3) Investigation of methods for the estimation of soil acidity.

1.—Determination of available potash and phosphoric acid.

The referee will furnish a sampling tube with which samples can be taken to a depth of 4 feet. The tube must be promptly returned to the Bureau of Chemistry, U. S. Department of Agriculture, or forwarded to another chemist, as may be directed, charges prepaid.

(a) Determination of potash dissolved by treatment with distilled water, following strictly the method of Moore,[a] except that distilled water is used as a solvent instead of N/200 hydrochloric acid.

It is not necessary to use acidified alcohol in washing the precipitate of potassium platinic chlorid, as ordinary 90 per cent alcohol serves the purpose just as well.

The referee has used this method on some 15 or 20 samples of soil and in all cases as much or more potash was obtained as was removed from the soil by the oat crop.

(b) Determination of available potash and phosphoric acid in N/200 hydrochloric.[a] Do not correct for basicity, but secure a uniform temperature of 40° C. and moderate agitation in Wagner machine.

The results secured last year on surface soils were not very satisfactory, but it is hoped that the estimation of the available material to greater depths will give better results.

2.—Methods for the analysis of alkali soils.

(a) Treat 10 grams of soils representative of different classes of alkali with 1 liter of distilled water, shaking frequently, and determine bicarbonates, carbonates, chlorids, and sulphates, after standing 12 hours, 24 hours, 48 hours, and 72 hours.[b]

(b) Treat 10 grams, 25 grams, and 50 grams of the soils used in (a) with 1 liter of distilled water with frequent shaking for the time giving the greatest solubility in (a) and determine bicarbonates, carbonates, chlorids, and sulphates.

The referee would impress upon those members of the association who have to deal with alkali soils the importance of having in our methods processes for the satisfactory estimation of the soluble salts in these soils. He would further urge that it is the duty of those so situated to work out and propose methods for this purpose. The referee therefore makes a personal appeal to each member in the alkali regions to devote some attention to this line of work this year in order that methods may be adopted, provisionally at least, for such cases.

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[a] J. Amer. Chem. Soc., **24**: 79.

[b] U. S. Dep. Agr., Bureau of Soils, Bul. No. 18.

3.—METHODS FOR THE DETERMINATION OF SOIL ACIDITY.

(Use Jena glassware for all work.)

As it may be inconvenient for many to secure suitable samples for this work, the referee will furnish samples to those who request them.

A.—Determination of acidity by the sodium chlorid method proposed by Hopkins, Knox, and Pettit.[a]

In the execution of this method note the following points:

(1) Determine as far as possible whether or not there is any appreciable amount of free strong acid in the sodium chlorid filtrate from the soil. This may be done by comparing the reaction of the filtrate with 5 per cent sodium chlorid solution to which known amounts of strong acid have been added, together with an indicator, such as methyl orange, and noting the sharpness and strength of color in the two cases.

(2) Note whether or not the end point is sharp, and state whether the titration was stopped when a very faint permanent pink color was secured, or continued until the color was strong.

(3) Note whether or not a precipitate is produced on adding the alkali to the boiling liquid; and if so, filter it off after titration, wash with water and determine what bases it contains, and estimate each accurately.

(4) Express all results on the basis of the water-free soil in parts of lime per million of soil.

B.—Determination of acidity by the limewater method proposed by Veitch.[b]

C.—Determine the reaction of the original soil furnished by the method proposed by the referee.[a]

DETERMINATION OF AVAILABLE POTASH AND PHOSPHORIC ACID.

Available potash and phosphoric acid were determined by the N/200 acid method, and available potash by simple treatment with water.

Potash and phosphoric acid were determined in different soil layers in samples drawn from the fertilized plots of the Maryland station. This soil is deficient in available phosphoric acid. The referee was unable to obtain soils to different depths which are known to be deficient in potash.

Most of the soils which have been worked on are drawn from the collection of the Bureau of Chemistry. The amount of potash and phosphoric acid removed from the soils by various crops is accurately known, thus affording an absolute standard with which to compare analytical results. I desire to express my great indebtedness both to Mr. Wiley and Mr. Moore for samples of soil and permission to transcribe their records. Both analytical and crop data are given in Table I.

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[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. 73, Proceedings Nineteenth Annua Convention, A. O. A. C., 1902, p. 114.

[b] J. Amer. Chem. Soc., 1902, **24**: 1120; Proceedings Nineteenth Annual Convention, A. O. A. C., 1902, p. 136.

TABLE I.—*Plant food removed by oats, by digestion in distilled water, and in N/200 acid (C. C. Moore and F. P. Veitch).*

[Parts per million.]

Description of sample.	Removed by oats.		Water soluble.	N/200 acid soluble.	
	K_2O	P_2O_5	K_2O	K_2O	P_2O_5
Vegetation experiment, Bureau of Chemistry:					
California soil, pot No. 1	26.0	.....	16.0	.....	.....
Illinois station soil, pot No. 5	57.0	.....	17.0	.....	.....
Massachusetts station soil, pot No. 12	27.0	.....	37.0	.....	.....
South Dakota station soil, pot No. 22	27.0	.....	18.0	.....	.....
California cultivated soil, pot No. 63	14.0	.....	21.0	.....	.....
Massachusetts cultivated soil, pot No. 78	34.0	.....	22.0	.....	.....
Michigan cultivated soil, pot No. 80	17.0	.....	17.0	.....	.....
Wisconsin cultivated soil, pot No. 90	28.0	.....	16.0	.....	.....
Pennsylvania cultivated soil, pot No. 129	40.0	.....	28.0	.....	.....
Cooperative Experiments, 1902:					
Wisconsin station soil (barnyard manure used)	38.0	35.0	27.0	30	64.0
Vermont station soil	22.0	17.0	32.0	56	1.5
Illinois station soil	34.0	9.0	33.0	31	2.0
Missouri station soil	30.0	7.0	34.0	42	2.0
Virginia station soil	62.0	31.0	137.0	217	18.0
Arlington station soil	15.0	3.0	20.0	41	2.0
North Carolina station soil	9.0	2.0	17.0	50	1.0
Florida station soil	7.0	4.0	12.0	20	5.0
Wyoming station soil	36.0	11.0	50.0	55	1.0
Rothamsted soils, barley continuously:					
Plot B_{1a}, only nitrogen applied	17.0	6.0	6.0	.....	.....
Plot B_{2a}, N and P_2O_5 applied	18.0	9.0	.....	.....	.....
Plot B_{3a}, N and K_2O applied	49.0	8.0	53.0	.....	.....
Plot B_{4a}, complete fertilizer applied	45.0	14.0	40.0	.....	.....
Rothamsted soils, wheat continuously:					
W_3, unmanured continuously	14.0	5.0	10.5	.....	.....
W_{10a}, only nitrogen applied	14.0	4.0	7.0	.....	.....
W_{11}, N and P_2O_5 applied	19.0	10.0	3.5	.....	.....
W_{13}, complete fertilizer applied	45.0	14.0	44.0	.....	.....
Hemp experiments, Bureau of Chemistry, Kentucky soil	36.0	4.0	23.0	120	2.0
Do	70.0	27.0	44.0	98	14.0
A. O. A. C. samples:					
No. 1, Ohio station, lacks P_2O_5	.....	.....	12.0	55	.6
No. 2, Pennsylvania station, lacks P_2O_5	.....	.....	33.0	111	1.5
No. 3, Maryland station, lacks P_2O_5	.....	.....	104.0	142	10.0
No. 4, Maryland station, well supplied with all	.....	.....	40.0	42	4.3
No. 5, Massachusetts station, lacks P_2O_5	.....	.....	65.0	175	.7
No. 6, Kentucky station, lacks K_2O	.....	.....	4.0	18	4.3
No. 7, Rhode Island station, lacks K_2O	.....	.....	2.0	12	1.3
No. 8, Rhode Island station, lacks P_2O_5	.....	.....	2.0	42	1.0
Maryland P_2O_5 experiments:					
Plot No. 10, 0 to 8 inches, lacks P_2O_5	.....	.....	3.2	29	.7
Plot No. 10, 8 to 16 inches	.....	.....	3.0	13	.5
Plot No. 10, 16 to 36 inches	.....	.....	2.0	16	.7
Plot No. 11, 0 to 8 inches, well supplied with all	.....	.....	1.0	25	5.0
Plot No. 11, 8 to 16 inches, well supplied with all	.....	.....	1.0	12	.7
Plot No. 11, 16 to 36 inches, well supplied with all	.....	.....	1.0	17	.7
Maryland K_2O experiments:					
Plot No. 4, 0 to 8 inches, well supplied with all	.....	.....	22.0	100	1.5
Plot No. 4, 8 to 16 inches, well supplied with all	.....	.....	18.0	166	1.5
Plot No. 4, 16 to 36 inches, well supplied with all	.....	.....	22.0	180	1.0

From these results it appears that the N/200 acid method when not corrected for bacisity extracts less phosphoric acid from most soils than is removed by crops, but usually dissolves more potash than oats remove. Could it be assumed that plants always feed to considerable depth, say 3 or 4 feet, the low solubility frequently shown would be amply sufficient for maximum crops. However, we have positive data showing that from a given weight of soil the oats obtained more phosphoric acid than the method shows to be available.

From the fact that this method, like most others, distinguishes quite sharply between fertilized and unfertilized plots, the indications are that acid of this concentration attacks chiefly the lime phosphates, while iron and alumina phosphates, as well as the phosphorus of organic matter, is apparently but little affected. For these reasons it appears desirable to study the solvent effect of more concentrated acids, at the same time endeavoring to reach the phosphorus in organic combination. For the same reason it is imperative to correct for basicity of the soil, otherwise only the solubility of calcium phosphate in salt solutions is obtained. A method is now being worked out for estimating the free mineral acid in the presence of acid salts, and it is hoped that valuable data will be obtained along this line. It is to be borne in mind in devising a method for available phosphoric acid that the activity of fungi may be reasonably expected to constantly place more phosphoric acid within reach of the higher plants, and it is practically impossible to prognosticate how much may be thus rendered available or to devise a chemical method that will measure it.

The potash declared available by this method appears to be usually greater than that removed by the oat plant, even under the most favorable conditions, and is much greater than is usually believed to be necessary for the production of maximum crops under field conditions. As potash, relatively speaking, is seldom the controlling element in our soils, it can not be assumed that the quantities shown by the method are not actually available, for other factors limit the amount removed by the plant.

The results on water-soluble potash have not generally borne out the indications of preliminary work with this solvent. The results, however, have sufficient significance to justify further work along this line, changing the ratio of solvent to soil from 5:1 to 10:1. One difficulty with this solvent is that it is often impossible to obtain a perfectly clear filtrate, but it has been found that the addition of acid for the purpose of flocculating the clay in the filtrate dissolves inappreciable quantities of potash from the clay.

Methods for the Analysis of Alkali Soil.

The referee was unable to do any work on this subject, and at his special request Mr. Seidell and Mr. Brown, of the Bureau of Soils, U. S. Department of Agriculture, undertook a study of the ratio of soil to water and of the effect of time on the solubility of alkali salts. The results obtained were reported by Mr. Seidell as follows:

Report on Analysis of Alkali Soils by A. Seidell.

The soil samples employed represented three classes of "alkali," characterized, respectively, by an excess of sulphates, carbonates together with chlorids, and chlorids.

In the experiments planned to show the effect of time upon the amounts of salts dissolved (first three analyses of each sample), three portions of each soil sample were used, one portion being for the one-day digestion period, another for the two-day, and a third for the three-day period. The weighed 5-gram portions of each sample were placed in flasks and 500 cc of water added, after which all of the flasks were shaken thoroughly for several minutes. After standing 24 hours the first set of three solutions was analyzed. The analysis consisted in siphoning off about 400 cc of the cloudy solutions, using 100 cc portions for the titrations, and, after clearing the remainder by means of several drops of hydrochloric acid, 100 cc portions were taken for the determination of sulphates.

The second set of flasks, after having stood 48 hours without additional shaking, were treated in the same manner as the first set. The third set were shaken well at the end of 48 hours and then allowed to stand 24 hours longer before being analyzed.

From the results obtained as above described it appears that in some cases there is a slight increase in the amount of soluble salts obtained by longer digestion, while in other cases there is a slight decrease. It therefore appears that no advantage results by extending the time of digestion beyond one day. It is possible, however, that if the experiments were modified so that the solutions were shaken at frequent intervals, or shaken continually by means of a suitable apparatus for different lengths of time, the amounts of salts dissolved would bear a definite relation to the lengths of time of the digestion.

The results obtained from the analysis of the solutions prepared with varying amounts of soil to the same amount of water show, on the other hand, in most cases a somewhat less percentage of soluble salts than are found when the amount of water to each gram of soil is greater. This fact is also brought out by the results of the analyses of alkali soils made by Mr. Breazeale and Mr. Veitch, and given in the report [a] of the referee on soils for last year.

It is to be noticed, however, that with alkali soils containing relatively large amounts of soluble salts the differences obtained by the use of 10 grams of soil to 1 liter of water and of 50 grams of soil to 1 liter are proportionately less than the differences found for soils containing very small amounts of readily soluble material.

It will also be seen that while the results presented last year indicate that the sulphate determination is most affected by the concentration of the solution, the results given in the accompanying table show that the sulphate determination is affected no more by change in the proportion of soil to water used than is the determination of chlorin or carbonates.

On the whole, it appears that while the use of less than 50 grams of soil to 1 liter of water yields higher results in most cases, especially those where the percentage of "alkali" is low, it is probably no great advantage to use the larger amount of water, since by keeping to the proportions already adopted in the Bureau of Soils it is very certain that all of the soluble material to which the "alkali" qualities of the soil are to be attributed are brought into solution and easily determined by the ordinary analytical processes.

The use of less soil than 50 grams per liter will no doubt cause some inconvenience and diminish the accuracy of the work in cases where the soil contains very small amounts of alkali, and therefore yields a very dilute solution in which the several determinations are to be made. There is probably no very great advantage to be attained in the use of any one of the ratios of soil to water here considered, either in respect to the facility of performing the analytical operations or the character of the results obtained. The laboratory of the Bureau of Soils has used the ratio of 50 grams of soil to 1 liter of water for four years, and in that time made several thousand analyses of alkali soils from all the principal alkali areas of this country and several areas in foreign countries.

In illustration of the points brought out in this report I submit herewith the analytical results obtained by myself and Mr. Bailey E. Brown, working independently.

TABLE II.—*Results on maximum solubility of alkali soils, and effect of time and variation of relative proportion of soil to water.*

[Results expressed in percentage of air-dried soil.]

ANALYSES MADE BY A. SEIDELL.

SAMPLE NO. 6603. (BILLINGS, MONT. ALKALI CRUST. TOTAL SALTS BY ANALYSES, 2.58 PER CENT.)

Time in days.	Ratio.		CO_3.	HCO_3.	Cl.	SO_4.
	Soil.	Water.				
	Grams.	*cc.*				
1	10	1,000	0.00	0.105	0.246	1.65
2	10	1,000	.00	.105	.246	1.64
3	10	1,000	.00	.099	.228	2.01
1	25	1,000	.00	.06	.14	1.6
1	50	1,000	.00	.05	.13	1.6
a1	50	1,000	.00	.03	.084	1.63

[a] Proceedings Nineteenth Convention of the A. O. A. C., 1902, p. 112.

TABLE II.—*Results on maximum solubility of alkali soils, and effect of time and variation of relative proportion of soil to water*—Continued.

SAMPLE NO. 6678. (WALLA WALLA COUNTY, WASH., ALKALI CRUST. EFFERVESCED WITH HYDROCHLORIC ACID. TOTAL SALTS, 22.54 PER CENT.)

Time in days.	Ratio.		CO_3.	HCO_3.	Cl.	SO_4.
	Soil.	Water.				
	Grams.	*cc.*				
1	10	1,000	2.918	3.15	11.26	2.25
2	10	1,000	3.57	2.12	11.47	2.49
3	10	1,000	3.27	2.72	11.50	2.60
1	25	1,000	2.86	3.51	11.47	2.37
1	50	1,000	3.34	2.06	10.69	2.25
[a]1	50	1,000	.....	.....	.....	2.35

SAMPLE NO. 7265. (SALT LAKE CITY, UTAH. 0–12 INS. EFFERVESCED WITH HYDROCHLORIC ACID. TOTAL SALTS, 1.64 PER CENT.)

1	10	1,000	0.074	0.181	0.897	0.177
2	10	1,000	.082	.166	.904	.164
3	10	1,000	.065	.215	.922	.156
1	25	1,000	.06	.10	.716	.200
1	50	1,000	.036	.066	.61	.198
[a]1	50	1,000	.034	.14	.56	.29

[a] Analyses made by Bailey E. Brown in the course of the regular routine of analytical work of the laboratory.

ANALYSES MADE BY B. E. BROWN.

SAMPLE NO. 6604.

Time in days.	Ratio.		CO_3.	HCO_3.	Cl.	SO_4.
	Soil.	Water.				
	Grams.	*cc.*				
1	1	100	0.00	0.24	0.175	1.67
2	1	100	.00	.24	.175	1.67
6½	1	100	.00	.24	.175	1.58

SAMPLE NO. 6676.

1	1	100	8.10	7.75	Trace.	1.68
2	1	100	7.83	8.05	Trace.	1.64
6½	1	100	8.16	7.14	Trace.	1.68

SAMPLE NO. 8033.

1	1	100	0.00	0.48	1.12	0.69
2	1	100	.00	.51	1.12	.68
6½	1	100	.00	.51	1.16	.67

The results are in the main in harmony with the results obtained last year except that the solubility of sulphates does not appear to be as much affected by concentration and time as was indicated by the figures obtained last year. This is doubtless due to the fact that none of the samples used this year contained relatively large amounts of soluble sulphates, the highest percentage being 2.60, while last year samples containing as high as 14.50 per cent were used.

The referee still thinks that the solubility of sulphates is influenced both by time and concentration. Indeed Cameron[a] has shown that under laboratory conditions

[a] U. S. Dept. of Agr., Bureau of Soils Bul. No. 18.

the maximum solubility of gypsum in sodium chlorid was not reached in much less than 72 hours. In sulphate solutions it would doubtless be slower. As calcium sulphate is a beneficial rather than an injurious compound, and in many cases the time of solution may be indefinitely prolonged, it is necessary to arbitrarily limit it and from 20 to 24 hours appears to be the most suitable period. It is regretted that those chemists who have to deal with the alkali problem did not participate in the work.

Soil Acidity.

The referee has devoted a great deal of time in the past three years to a study of soil acidity and methods for its estimation. Some of the statements made are based on work the details of which will be published elsewhere. The work has been confined to the limewater method and the sodium-chlorid method. In the first work done with the sodium-chlorid method it was at once observed that in titrating the solution with alkali a precipitate was always formed. This was usually found to be aluminum hydrate, and careful investigation showed that this precipitate calculated to aluminum oxid required practically all the alkali used in the titration, assuming that a solution of aluminum chlorid was being titrated. The figures obtained are given in Table III.

Table III.—*The relation between alkali required and dissolved acid-salt-forming bases.*

Description of sample.	Amount of N/20 alkali required by—			Bases in 100 cc alkali filtrate.		Amount of N/20 alkali required by—		
	1. 100 cc sodium chlorid filtrate.	2. 100 cc water filtrate.	3. 100 cc sodium chlorid sol. acids.	4. Ferric, aluminum, and manganese oxids.	5. Zinc oxid.	6. Column 4, expressed as aluminum oxid.	7. Zinc oxid.	8. Not accounted for by aluminum and zinc oxids.
	cc.	*cc.*	*cc.*	*mg.*	*mg.*	*cc.*	*cc.*	*cc.*
Fine sand, rich in organic matter	3.7	2.7	1.0	1.4	.....	1.5	.....	2.7
Rhode Island station, very acid	1.6	1.0	.6	2.0	.....	2.0	.....	0.0
Kaolin, good grade	1.6	.7	.9	1.2	.....	1.3	.....	.3
Kaolin, good	4.2	Not det.	.....	3.0	.....	3.3	.....	.9
Vegetation experiments, Bureau Chemistry, California soil:								
Limed	5.3	1.0	4.3	2.2	8.6	2.4	3.6	0.0
Not limed	19.0	3.4	15.6	2.6	29.4	3.0	12.2	3.8
Vegetation experiments, Bureau Chemistry, Illinois station	2.2	Alkaline.	2.2	1.0	.....	1.1	.....	1.1
Carlyle silt loam, subsoil, Illinois	31.5	Not det.	.....	30.4	.....	33.4	.....	0.0
Plot No. 6, fertilizer experiments, Pennsylvania station	.6	Alkaline.	.....	.6	.....	.6	.....	0.0
Plot No. 10, phosphoric acid experiments, Maryland station	1.2	.....	.....	.5	.....	.6	.....	.6
Sand from propagating house.	25.7	24.0	1.7	28, Fe_2O_3	.....	21.5	.....	4.2
Collington loam, glauconitic, Prince George County, Md.	22.6	.....	.....	22.8	.....	23.5	.....	0.0

TABLE III.—*The relation between alkali required and dissolved acid-salt-forming bases*—Continued.

Description of sample.	Amount of N/20 alkali required by—			Bases in 100 cc alkali filtrate.		Amount of N/20 alkali required by—		
	1. 100 cc sodium chlorid filtrate.	2. 100 cc water filtrate.	3. 100 cc sodium chlorid sol. acids.	4. Ferric, aluminum, and manganese oxids.	5. Zinc oxid.	6. Column 4, expressed as aluminum oxid.	7. Zinc oxid.	8. Not accounted for by aluminum and zinc oxids.
	cc.	*cc.*	*cc.*	*mg.*	*mg.*	*cc.*	*cc.*	*cc.*
Loam, District of Columbia, very acid, growth only sorrel	22.8	.....	.....	21.8	.....	23.0	.....	0.0
Clay subsoil, Piedmont region.	2.8	.....	.....	1.8	.....	2.0	.....	.8
Fine sand, rich in organic matter, treated with—								
Normal sodium chlorid	3.6	.7	2.9	2.3	.....	2.5	.....	1.1
Double normal sodium chlorid	5.2	.7	4.5	3.5	.....	3.9	.....	1.3
Normal potassium chlorid.	6.6	.7	5.9	5.0	.....	5.0	.....	1.1
Double normal potassium chlorid	8.0	.7	7.3	6.9	.....	7.6	.....	.4
Brick clay treated with—								
Normal sodium chlorid	22.0	.4	21.6	17.8	.....	19.1	.....	2.9
Double normal sodium chlorid	25.3	.4	24.9	20.0	.....	22.0	.....	3.3
Normal potassium chlorid.	27.6	.4	27.2	20.6	.....	22.2	.....	5.4
Double normal potassium chlorid	29.6	.4	29.2	21.6	.....	23.2	.....	6.4

These results show conclusively that the apparent acidity of the solution is due to the presence of an acid salt, usually aluminum chlorid. In no instance was the referee able to find an appreciable amount of free hydrochloric acid, even in the most acid of these solutions. The test used was that previously given and its applicability here can be easily proved. Solutions of other salts and other strengths of sodium chlorid have also been tried, and the results of these experiments are given in Table IV.

TABLE IV.—*Sodium-chlorid method; acidity by different solvents of varying strength.*

Description of sample.	Soil.	Kind and strength of solvent.	N/20 alkali to 100 cc of filtrate.	Acidity, parts per million.
	Grams.		*cc.*	
Brick clay (moist sample)	200	5 per cent sodium chlorid	28.0	980
			29.0	1,015
Do	200	10 per cent sodium chlorid	40.0	1,400
Do	200	20 per cent sodium chlorid	40.0	1,400
Do	100	5 per cent sodium chlorid	19.2	1,344
			19.0	1,300
Do	100	10 per cent sodium chlorid	25.0	1,750
			26.0	1,820
Do	100	20 per cent sodium chlorid	25.5	1,785
			26.5	1,855
Do	100	5 per cent sodium sulphate	18.8	1,316
Do	100	5 per cent potassium sulphate	22.4	1,568
			23.0	1,610

TABLE IV.—*Sodium-chlorid method; acidity by different solvents of varying strength*—Continued.

Description of sample.	Soil.	Kind and strength of solvent.	N/20 alkali to 100 cc of filtrate.	Acidity, parts per million.
			cc.	
Brick clay (air-dry sample)	100	Normal sodium chlorid	22.0	1,540
			23.0	1,610
Do	100	2 normal sodium chlorid	25.3	1,771
Do	25	Normal sodium chlorid	7.5	2,100
Do	50	Normal sodium chlorid	13.5	1,890
Do	100	Normal potassium chlorid	27.6	1,932
Do	100	2 normal potassium chlorid	29.6	2,072
Sandy soil, rich in organic matter	100	Normal sodium chlorid	3.6	252
Do	100	2 normal sodium chlorid	5.2	364
Do	25	Normal sodium chlorid	1.7	476
Do	50	Normal sodium chlorid	2.5	350
Do	100	Normal potassium chlorid	6.6	462
Do	100	2 normal potassium chlorid	8.0	560

The acidity appears to vary with the kind and strength of the salt solution, but the variation can not be foretold nor is the assumption justified that any particular strength or kind of a salt solution is a measure of the lime requirements of soils. The originators of the method found that the reaction taking place was not complete, and proposed a factor of 1.5 and later of 2 to correct for this condition. It was found that the brick clay reported in Table IV required the factor 1.3, while the sand rich in organic matter required a factor of 2. It is evident, therefore, that the same factor is not applicable to all soils and that a large error is introduced by the use of a single factor. The determination of the factor for every soil would lengthen the method greatly.

It has also been found that a soil containing organic matter, limed in accordance with the results obtained by this method, is seldom neutral but still is acid, according to the method. Some faintly alkaline soils also show acidity by this method, as is seen in Table V.

It is concluded that this method does not show the acidity due to water insoluble organic acids, that there is no quantitative setting free of hydrochloric acid by such organic acids, and that the apparent acidity as thus determined is due to the water soluble acids and acid salts plus the acid salts which are produced by the action of the salt solution upon the hydrous or other silicates of the soil. The method in its present form has nothing like the accuracy claimed for it by the originators. If the acidity requires 20 or more cubic centimeters of N/20 alkali per 40 grams of soil, duplicates may differ from one-half to 1 cc of alkali, which is equal to 35 to 70 parts per million, and the multiplication of this error by an erroneous factor may increase the actual error to as much as 600 parts per million.

Several modifications of the limewater method have been tried, the results of which are given in Table V. No modification presented any advantage over the original method and the table calls for no detailed discussion:

TABLE V.—*Acidity expressed as parts of calcium oxid per million of soil.*

Description of sample.	Sodium chlorid method.	Calcium hydroxid method.			
		Dried on bath.		Dried in air; stood 16 hours.	Not dried; stood 16 hours.
		Stood 16 hours.	Stood 40 hours.		
Sandy soil rich in organic matter, Norfolk, Va	258	4,000	3,400	3,900	3,500
Sandy soil rich in organic matter, after cropping	252	3,900	3,200	>3,400	2,100
Sandy soil rich in organic matter, limed sodium chlorid method 200 parts per M	161	3,800	2,700	.....	.....
Sandy soil rich in organic matter, limed 1,700 parts per M	49	2,400	1,500	<1,900	800
Loam, very acid, growth sorrel	1,365	.....	2,200	.....	2,500
Loam, very acid, growth sorrel, after cropping	1,631	2,400	2,000	<2,000	2,000
Loam, very acid, growth sorrel, after cropping, limed 1,200 parts	658	2,100	1,200	.....	1,600
Loam, very acid, growth sorrel, after cropping, limed 2,250 parts per M	91	1,200	600	<1,000	600
Pot No. 67, P_2O_5 experiments, calcium oxid series, Rhode Island station	112	1,000	600	> 800	800
Pot No. 67, vegetation experiments, Bureau of Chemistry, California soil, limed 1,200 parts	370	900	.....	.....	.....
Pot No. 68, vegetation experiments, Bureau of Chemistry, California soil, unlimed	1,330	2,000	2,000	1,900	1,600
Pot No. 35, vegetation experiments, Bureau of Chemistry, Illinois station	153	(a)	.....	.....	.....
Kaolin No. 1	112	100	100	100	100
Kaolin No. 2	280	150	.....	.....	.....
Carlyle silt loam, Illinois	2,170	2,600	<2,400	2,600	2,400
Coarse sand from propagating house	1,800	1,000	.....	.....	.....
Collington loam, subsoil, glauconitic, Maryland	1,470	1,100	.....	.....	.....
Brick clay, 4 feet below surface, Maryland	1,610	1,400	.....	<1,300	1,200
Calcium oxid plot, 10 bushels per acre, 0 to 6 inches, Maryland station	196	500	.....	< 400	300
Calcium oxid plot, 20 bushels per acre, 0 to 6 inches, Maryland station	63	300	.....	.....	200
Calcium oxid plot, 30 bushels per acre, 0 to 6 inches, Maryland station	28	(a)	.....	.....	.....
Calcium oxid plot, 0 bushels per acre, 0 to 6 inches, Maryland station	406	600	.....	< 500	400
Plot No. 4, "soil test" plot, Rhode Island station, taken 1894	518	1,800	.....	<1,700	1,700

a Alkaline.

The fact that long standing of the treated and dried soil in contact with water shows less acidity than short standing is interesting and seems to indicate the breaking up of the more insoluble calcium silicates on long standing. The method is rather long and cumbersome and for this reason is objectionable. It is accurate to within 300 to 400 parts per million under extreme conditions, i. e., where the supernatant liquid is highly colored by organic matter and where the acidity is high. On soils of low acidity which give colorless, clear liquids the results are accurate to within 100 parts per million. Both methods are simple in their details and it does not appear worth while to devote further analytical study to them until a more definite knowledge is possessed as to the actual lime requirements of soils as a basis for such investigations.

RECOMMENDATIONS.

It is recommended—

(1) That in the continuation of the work on methods for available phosphoric acid the referee compare greater concentrations than N/200 acid.

(2) That the work on the solubility of phosphoric acid and potash in the different soil layers be continued.

(3) That the referee investigate methods for determining total phosphoric acid.

(4) That the following provisional method for the analysis of the water soluble portion of alkali soils be incorporated in the methods:

PROPOSED PROVISIONAL METHOD FOR THE DETERMINATION OF WATER SOLUBLE CONSTITUENTS OF ALKALI SOILS.

(a) *Preparation of solution.*

Place 25 grams of soil in a graduated 500 cc flask, fill to the mark with distilled water, allow to stand twenty to twenty-four hours, shaking frequently, and filter through a dry filter, or if clear, siphon off the supernatant liquid.

(b) *Determination of total carbonates.*

Titrate an aliquot portion of this filtrate with N/20 sulphuric acid, using a few drops only of methylorange as indicator, and be very careful not to run over the end point. From the number of cubic centimeters of acid required calculate the total CO_2 as carbonates.

(c) *Determination of chlorin.*

To the aliquot portion used for the titration of carbonates add a few drops more of N/20 acid, then a little potassium chromate, and titrate with N/10 silver nitrate and calculate to chlorin (cc N/10 silver nitrate multiplied by 0.003518=the number of grams of chlorin).

(d) *Determination of sulphuric acid.*

An aliquot portion of the original filtrate is made slightly acid with hydrochloric acid, and if clay is flocculated the solution is filtered and the filter washed free of sulphates. To the boiling solution add slowly a large excess of barium chlorid solution and finish the determination as in 4 (j)[a]. In solutions containing large amounts of carbonates the ignited barium sulphate should be treated with hydrofluoric acid and a few drops of sulphuric acid ignited and again weighed.

(e) *Determination of bases.*

If the original filtrate is turbid, as it is apt to be when the soil contains carbonates and but little of other salts, it may be cleared by adding 5 cc of thick alumina cream to 200 or 300 cc and filtering through a dry filter. In using portions of this filtrate for the determinations, correction should be made for the added alumina cream. To aliquot portions of the solution add hydrochloric acid in excess, and in cases where silica may be present, separate in the usual way and determine the bases as in 4 (b), (c), (d), and (e).[b]

(f) *Determination of phosphoric acid.*

Add an excess of nitric acid to an aliquot portion of the original solution. Make nearly neutral with ammonia, precipitate with ammonium molybdate, finishing the determination as in 4 (g).[c]

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[a] U. S. Dept. of Agr., Bureau of Chemistry Bul. No. 46, Methods of Analysis, p. 75.
[b] Ibid., pp. 72, 73.
[c] Ibid., p. 74.

(5) That on page 72, under 4 (a), "Acid digestion of the soil," the tenth line be changed to read, "filter, wash free of chlorids, and again evaporate the filtrate to complete dryness as before."

(6) That on page 74, under 4 (g), determination of phosphoric acid, the official method given be marked "(a)" and the following optional provisional method be inserted as (b):

(b) *Optional provisional method:* Proceed as in (a) "until all the phosphoric acid is precipitated" and then finish the determination as follows:

After standing for three hours at a temperature not above 50°, filter on a small filter, wash with water until two fillings of the filter do not greatly diminish the color produced with phenolphthalein by one drop of standard alkali. Place the filter and precipitate in the beaker and dissolve in standard alkali, add a few drops of phenolphthalein solution, and titrate with standard acid, 1 cc of which equals 0.0005 gram of phosphoric acid (P_2O_5).

(7) That on page 74, 4 (h), the word "available" be omitted from the heading "Provisional method for the determination of available phosphoric acid."

(8) Under 4 (k), "Determination of potash and soda," insert in sixth line, after "hot water," Finish by (1) or (2).

(1) Add ammonia, etc. (present method).

(2) To the hot filtrate add slowly a few cubic centimeters of ammonium sulphate (75 grams salt per liter) and after the precipitate has settled filter and wash thoroughly with hot water, evaporate the filtrate to dryness, and ignite. Add a little hot water, and if any material is not dissolved filter, wash, evaporate to dryness and ignite as before. Add a little ammonium carbonate, volatilize to break up bisulphates. Heat to redness and weigh as sulphates.

(9) That on page 76, paragraph 10, "Determination of humus," eleventh line, insert after the word "filtered" "the filtrate must be perfectly clear and free from turbidity."

(10) That the retiring referee be instructed to make such verbal corrections as these changes will involve and submit them to the secretary for approval and publication.

Mr. Lipman. I have a paper on soils to submit.

THE FIXATION OF ATMOSPHERIC NITROGEN BY BACTERIA.

By J. G. Lipman.

Important discoveries in the domain of agricultural bacteriology have often been preceded by repeated failure. No better example need be cited than the history of that well-known problem, the fixation of atmospheric nitrogen by the joint activity of *Bacillus radicicola* and of leguminous plants. One need but recall the work of Boussingault,[a] and the work of Lawes, Gilbert, and Pugh,[b] and their painstaking care and the conscientious attention to detail, to realize the limitations of even the best of investigators. Their failure is no less instructive than the partial success of Atwater[c] and the complete success of Hellriegel and Wilfarth.[d] Both the failure and the success lay a strong emphasis on the importance of the biological properties of arable soils as a factor in the economy of plant growth. It is a significant fact, and not devoid of encouragement to those who are interested in the progress of agriculture, that the soil chemist and the soil physicist have come to a better appreciation of

[a] Compt. rend., 1859, 48: 303; Agronomic, etc., 1860, 2d ed., vol. 1.

[b] Philosophical Transactions, 1861, part II; Philosophical Transactions, 1888.

[c] Amer. Chem. J., vol. 6, No. 6; vol. 8, No. 5.

[d] Beilag. Zeit. Ver. Rübenzucker-Ind. d. d. R., 1888.

their common aims and have learned to work together. And it is to be hoped that in the study of soil problems the proper place will be accorded to soil bacteriology. Nothing but good can result from this recognition. It will be found that soil bacteriological studies will help to explain many a seemingly inexplicable result obtained in field and vegetation experiments.

The fixation of atmospheric nitrogen by soil bacteria in cooperation with higher plants has been the subject of much thought and of much study, and we all know what the achievements in this field of research have been; but as to the fixation of elementary nitrogen by bacteria independently of higher plants very little is known. The collection of facts bearing on this point is of recent origin, and most of these facts have been brought to light within the last two years. A rapid survey of the subject and its historical development will show its present status.

In his second series of experiments on the fixation of atmospheric nitrogen Boussingault obtained results which showed a gain of nitrogen in the soils experimented with. He grew leguminous plants in a mixture of rich garden soil and sand, and found at the end of the experiment a gain of 71.4 mg of nitrogen on the 359.7 mg supplied in the seed and soil. That was in 1858. In the following year he repeated the experiment, and obtained a gain of 67.1 mg on a total of 338.0 mg of nitrogen supplied in the soil and in the seed. Stranger still, he noted that there was more gain in the soil itself than in the plants; that the soil, moreover, gained not only in nitrates and ammonia, but organic matter also, possibly the remains of living organisms. To use his words as transcribed by Gilbert:

Vegetable earth contains not only dead organic matter, but living organisms—germs—the vitality of which is suspended by drying and reestablished under favorable conditions as to moisture and temperature. This mycodermic vegetation is not always visible to the naked eye and its progress must be followed by the aid of the microscope. The mycoderms have only an ephemeral existence, and they leave their detritus in the soil, which in time may give rise to ammonia and to nitric acid. Even if the nitrogen of the air takes part in nitrification, a part of the nitrogen will exist in mycoderms and their remains.

Evidently Boussingault had in his hand facts which might have revealed to him the cause of these gains had contemporary information been sufficient to throw greater light on the subject. Hellriegel and Wilfarth's discovery of symbiotic fixation was still almost thirty years away, Berthelot's study of nitrogen-fixing bacteria, Winogradsky's isolation of nitrifying bacteria and of the nitrogen-fixing *Clostridium pasteurianum* were not to come for more than three decades, and in fact the technique of modern bacteriology was practically unknown. Small wonder, then, that, clear sighted as he was, Boussingault failed to recognize the significance of his analytical results. He deliberately eliminated them from his later studies, and placing more reliance on his later experiments (1860–1871), he wrote to Gilbert in 1876:

Quant a l'absorption de l'azote gazeux de l'air par la terre végétale je ne connais pas une seule observation irréprochable qui l'etablisse; non seulement la terre n'absorbe pas d'azote gazeux mais elle en émet, ainsi que vous l'avez reconnu avec Mr. Lawes, comme l'a vu Reiset pour le fumier, comme nous l'avons constaté, M. Schloesing et moi, dans nos recherches sur la nitrification. S'il est en physiologie un fait parfaitement démontré, c'est celui de la non assimilation de l'azote libre par les végétaux et je puis ajouter par les plantes d'un ordre inferieur, telles que les mycodermes, les champignons.

Gilbert comments on this as follows:

It is remarkable that in that letter he should so expressly give his opinion against the supposition that the lower organisms within the soil affect the fixation of free nitrogen, notwithstanding the evidence of his experiments of 1858 and 1859 that the gain, if there really were gain, was chiefly by the soil, and chiefly as organic matter, the accumulation of which he attributed to the development of mycodermic vegetation. It is true that in the discussion of the results he did not give any clear indication whether he considered that the apparent fixation was due in the first instance to the process of nitrification, the mycoderms only appropriating the nitrogen of the

nitrates formed, or whether he supposed that the mycoderms themselves were the primary agents, and that the nitrification was only the result of the oxidation of the mycodermic remains.

It did, indeed, seem that in the results in question there was *the germ of the germ explanation* of the fixation of free nitrogen, if such took place at all, in connection with vegetation. But we confess that Boussingault's very distinct conclusion against the assumption of any such agency, notwithstanding the indications of some of his own experiments, leads us still to ask further confirmation of the evidence of others in the same direction, which has been accumulating during the last few years.

Gilbert made the above statement in 1888, thirty years after Boussingault had made the observation in question. He, too, was still inclined to doubt that there might be fixation of nitrogen in arable soils, notwithstanding the fact that Berthelot[a] had already a considerable amount of analytical data showing that under certain conditions bare soils can increase their content of combined nitrogen at the expense of the free nitrogen of the air. Working with argillaceous sand, with clay, and with crude kaolin, he obtained[b] in six months (April to October, 1885) an increase in every case, the greatest gain being made by the argillaceous sand. When the soil was placed in a large 4-liter flask and sterilized no gain was observed, while a soil similarly treated but not sterilized showed a gain of nitrogen. At certain intervals samples of the soil were taken and analyzed, the analyses showing a gradual and steady increase in the total combined nitrogen, while the amount of nitrate produced was small and the slight amount of ammonia originally present either remained constant or tended to decrease. The results thus obtained indicate that the nitrogen gained by the soil was in the insoluble organic form, and this, taken together with the fact that the gains were made in unsterilized vessels and largely during the warmer part of the year, justifies the assumption that the fixation was accomplished by living organisms. Without going into further detail, it is sufficient to state here that corroborative evidence as to the fixation of nitrogen by soils and soil organisms was also furnished by Joulie,[c] Dietzell,[d] Tacke,[e] Gautier and Drouin,[f] Schloesing and Laurent,[g] Frank,[h] Koch and Kossowitch,[i] Kossowitch,[j] Krüger and Schneidewind,[k] Richter,[l] Petermann,[m] Immendorff,[n] Deherain,[o] Pagnoul,[p] Pickard,[q] Breal,[r] and Kühn.[s] Not content with proving that there may be an accumulation of nitrogen in bare and uncropped soils, Berthelot[t] also attempted to isolate the specific organisms to which such fixation is due. With the aid of Professor Guignard he made inoculations into sterile bouillon from a garden soil, and after incuba-

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[a] Chimie Vegetale et Agricole, Paris, 1899.
[b] Compt. rend., **101**: 775; Idem, **104**: 209, 630.
[c] Bul. de la Soc. des Agric. de France, 1886, No. 1, pp. 19–29.
[d] Naturforscher. Versaml., Magdeburg, 1884.
[e] Landw. Jahrb., 1889, **18**: 439.
[f] Compt. rend., 1888, **106**: 570; Idem, 1892, **115**: 572.
[g] Compt. rend., 1888, **106**: 1607.
[h] Landw. Jahrb., 1888, **17**: 421; 1890, **19**: 588.
[i] Bot. Ztg., 1893, **51**: 321.
[j] Bot. Ztg., 1894, **52**: 97.
[k] Landw. Jahrb., 1900, p. 771.
[l] Landw. Versuchs-Stat., 1899, **51**: 221.
[m] Abstr. Chem. Centr., 1893, **1**: 988.
[n] Landw. Jahrb., 1892, **21**: 281.
[o] Compt. rend., 1897, **125**: 278.
[p] Ann. agron., 1890, **16**: 250; Abstr. Ex. Sta. Rec., **3**: 120.
[q] Compt. rend., 1892, **114**: 81.
[r] Ann. agron., 1892, **18**: 269; Abstr. Ex. Sta. Rec., **4**: 375.
[s] Abstr. Centr. f. Bact., pt. 2, **7**: 601.
[t] Compt. rend., 1893, **116**: 842.

tion at 20° and proper dilution with sterile bouillon gelatin plates were prepared. Inoculations were made from the colonies which appeared on the plates, and the bacteria thus isolated were tested for their nitrogen-fixing power. A careful description of these organisms is lacking, so that their identification is impossible. Berthelot gives, however, some general statements concerning them, and, what is more important, shows by his analytical results that there was a gain of combined nitrogen in some cases. He concludes from these experiments that there are chlorophylless bacteria in the soil capable of fixing elementary atmospheric nitrogen. They require organic carbon and hydrogen and enough combined nitrogen to promote their initial growth. When the amount of combined nitrogen present becomes considerable, the bacteria prefer to cover their nitrogen requirements by utilizing the combined soil nitrogen, and for this reason the fixation of soil nitrogen has its limits. It is quite evident from the above that Berthelot had cleared the ground for more exact bacteriological studies. To him belongs the credit of having proved conclusively that the fixation of gaseous atmospheric nitrogen may be accomplished in arable soils by virtue of certain micro-organisms contained in them. His skill as a chemist enabled him to overcome the many difficulties involved in the analytical work and to render his researches of permanent interest.

At this point the problem was attacked by Winogradsky,[a] whose admirable mental equipment made it possible for him to shed much light on the subject. His first communication dates back to 1893 and deals with experiments that were carried out with impure cultures of a butyric ferment. The nutritive solutions employed by him were carefully freed from the last traces of nitrogen, and contained besides the mineral salts a fermentable organic compound, dextrose. Butyric fermentation was produced in these solutions by a long spore-bearing bacillus, which developed normally in the absence of combined nitrogen. Winogradsky had not succeeded at this time in isolating this bacillus in pure culture. In the above experiments it was accomplished by two other bacilli. It seemed, however, that the latter took no part in the fixation of free nitrogen, for they refused to grow in media free from nitrogen, but grew vigorously in solutions to which small quantities of ammonium salts were added. This, taken together with the fact that they produced neither gas nor butyric acid, led Winogradsky to believe that these accompanying bacteria were incapable of fixing free nitrogen, although they were capable of growing in solutions very poor in combined nitrogen. At the end of the experiment the solutions were evaporated to dryness under diminished pressure and analyzed according to the Kjeldahl method. It was found that the maximum fixation was attained where no combined nitrogen was purposely added, namely, with 3.1 mg of nitrogen for every gram of dextrose used. When combined nitrogen was added to the culture solution, the fixation of nitrogen was diminished. Thus, with 2 grams of dextrose and 1.8 mg of combined nitrogen in the solution there were 1.7 mg of nitrogen fixed, while with 4 grams of combined nitrogen added the fixation was only 0.6 mg of nitrogen.

In a later communication Winogradsky[b] reports experiments with pure cultures of the butyric ferment. He isolated them by anaerobic culture methods, and found that aerobic growth takes place only in the presence of certain aerobic bacteria, which serve to diminish the oxygen pressure. With the two accompanying bacteria mentioned elsewhere and the butyric ferment he noted a diminution in the amount of nitrogen fixed as the concentration of the dextrose solution or the amount of initial nitrogen increased. When the amount of combined nitrogen added reached 21.2 mg for every gram of dextrose, there was even a loss of nitrogen (2.2 mg). Winogradsky concludes from these experiments that the gain of nitrogen depends on the proportion of the combined nitrogen to the sugar. In order that any gain

[a] Compt. rend., 1893, **116**: 1385.
[b] Compt. rend., 1894, **118**: 353.

may be obtained the proportion of combined nitrogen to the dextrose should be 6:1,000. Because of the characteristic formation of *Clostridia*, Winogradsky named this bacillus *Clostridium pasteurianum*.

The researches of Berthelot and Winogradsky soon led to attempts at the practical development of the subject, and this received a decided impetus when Caron, of Ellenbach, Germany, made public the results of his investigations.[a] These soon attained considerable notoriety and led to the commercial exploitation of his culture. The firm of Friedrich Bayer & Co., of Elberfeld, Germany, placed on the market a bacterial preparation under the name of Alinit. This preparation, the manufacturers claimed, would enable nonleguminous crops to make use of free atmospheric nitrogen provided that certain precautions be observed.

These claims led to an extended study of Alinit by men like Stoklasa,[b] Lauk,[c] Stutzer and Hartleb,[d] Kolkwitz,[e] Gain,[f] Lutoslawski,[g] Schultze,[h] Kruger and Schneidewind,[i] and Sewerin.[j] A careful study of the evidence submitted by these investigators shows that *B. ellenbachensis* of the Alinit preparation is incapable of fixing atmospheric nitrogen. Attempts were also made by Frank,[k] Immendorff,[l] Gonnermann,[m] and others to determine whether *B. radicicola* is capable of fixing atmospheric nitrogen, when developing outside of the legume tubercles, but with doubtful[n] or negative results, while Maze,[o] on the other hand, claims that he succeeded in obtaining positive results.

Then came Beijerinck's communication in 1901,[p] dealing with a new group of bacteria. Space will hardly permit a lengthy review of this statement and the subsequent report by him. Suffice it to say that he gave the name of Azotobacter to the entire group, and described two members *A. chroococcum* and *A. agilis*. The former was found to occur in most cultivated soils, the latter in the canal water at Delft. These two, together with *A. vinelandii* isolated by the writer from a Vineland soil in south Jersey, constitute, in so far as I am aware, all the known members of this interesting group of bacteria. Beijerinck, together with Van Delden,[q] found that *A. chroococcum* can fix only very slight quantities of nitrogen, if any at all, when growing in pure culture, and that growth stops before the carbon source is exhausted. Large amounts of nitrogen are fixed only when it is growing in symbiosis with other organisms. Gerlach and Vogel,[r] on the other hand, isolated an organism which they found capable of fixing considerable amounts of atmospheric nitrogen when growing in pure culture. They believe that the organism isolated by them is identical with Beijerinck's *A. chroococcum*.

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[a] Landw. Versuchs-Stat., 1895, **45**: 401.
[b] Centr. f. Bact., etc., 1898, pt. 2, **4**: 39, 78, 119, 284, 507, 535.
[c] Idem, p. 290.
[d] Idem, pp. 31, 73; Idem, 1899, pt. 2, **5**: 706; Bot. Centr., **72**: 229.
[e] Centr. f. Bact., etc., pt. 2, 1899, **5**: 670.
[f] Rev. gen. de botaniq., 1899, **11**: 18–28; Centr. f. Bact., etc., 1899, pt. 2, **5**: 847.
[g] Deut. Landw. Presse, 1898, **25**: 920.
[h] Landw. Jahrb., 1901, **30**: 319.
[i] Landw. Jahrb., 1899, **28**: 579.
[j] Centr. f. Bact., etc., 1902, pt. 2, **9**: 712, 746.
[k] Bot. Ztg., 1893, **51**: 139.
[l] Landw. Jahrb., 1892, **21**: 218.
[m] Landw. Jahrb., 1894; Jacobitz, Centr. f. Bact., etc., pt. 2, **7**: 783, 883, 876.
[n] Beijerinck, Bot. Zt., **46**; Jacobitz, Centr. f. Bact., etc., pt. 2, **7**: 783, 883, 876.
[o] Annal. Inst. Pasteur, 1898, **12**: 1, 128; Abst. Ex. Sta. Rec., 1898, **10**: 318.
[p] Centr. f. Bact., etc., pt. 2, **7**: 561.
[q] Idem, 1902, pt. 2, **9**: 3.
[r] Idem, 1902, pt. 2, **8**: 669; 1903, pt. 2, **10**: 636.

The researches of Beijerinck and Van Delden, of Gerlach and Vogel, and of Winogradsky,[a] were carried out with specific bacteria which have been described sufficiently to allow their recognition by others. Their work shows that there are at least two classes of bacteria capable of fixing large quantities of nitrogen, namely, spore-forming anaerobic and nonspore-forming aerobic organisms. Practical attempts to decide whether they can be used to increase the fertility of the soil are wanting if we except the investigations of Gerlach and Vogel, and of Freudenreich.[b] But from the theoretical standpoint we have to deal here with a group of very interesting bacteria. In some still unexplained way they can break up the molecules of elementary nitrogen and cause it to combine with other elements to form ultimately the albuminoids of the bacterial body. It is not known whether the albuminoid molecules are the first product of the synthesis or not, but it is believed that simpler nitrogenous substances are first formed, although these have thus far escaped detection. Certain it is that the solution of this problem will help us to understand the synthetical processes, not only of the bacterial cell, but all the synthetical processes in the cells of higher plants.

The Isolation of Nitrogen-Fixing Bacteria of the Azotobacter Group.

The solutions employed for the purposes of isolation had the following composition:

Tap water	cubic centimeters	1,000.00
Mannite	grams	20.00
Potassium phosphate (K_2HPO_4)	gram	.5
Magnesium sulphate	do	.2
Calcium chlorid	do	.02
Potassium nitrate	do	.005

The whole was made neutral to phenolphthalein with sodium hydrate, and after sterilization in the autoclave at 1.5 atmospheres pressure was inoculated with a small quantity of fresh soil. The crude culture thus obtained by inoculation with a Vineland soil was reinoculated into a similar culture containing 20 grams of glycerin in place of the mannite. In ten days reinoculations were made into fresh glycerin solutions, and from these stroke cultures were made on mannite agar. In 3 days at 28° there appeared on these plates, among others, whitish, dense, raised colonies. Microscopical examination showed them to consist of azotobacter bacteria, and inoculation was accordingly made into glucose solutions, composed like the others, but with glucose instead of mannite or glycerin. Evidence of vigorous growth became apparent in 3 or 4 days, and the microscopical examination showed an apparently pure culture of organism occurring as short rods or large cocci, with many of the former actively motile. These were replated three times on mannite agar, and in each case macroscopical as well as microscopical examination showed only one kind of colonies and one kind of organisms. Inoculation into meat extract bouillon left the latter clear, although a very slight precipitate was formed here. Microscopical examination also here showed the presence of the same organism. The cultural characteristics in various media showed it to be essentially different from the organisms described by Beijerinck, and it was therefore named *A. vinelandii*.

In order to show its behavior in nitrogen-free or nitrogen-poor solutions, the experiments following are presented.

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[a] Centr. f. Bact., etc., 1902, pt. 2, **9**: 43. [b] Idem, pt. 2, **10**: 514.

SERIES VI.—*100 cc glucose solution.*

No. 1, size of flask, 250 cc, sterile.
No. 2, size of flask, 250 cc, inoculated.
No. 3, size of flask, 500 cc, inoculated.
No. 4, size of flask, 1,000 cc, inoculated.

After 10 days the cultures were analyzed with the following results:

No.	Ammonium hydrate (0.003038 gram nitrogen per cubic centimeter).	Nitrogen found.	Nitrogen fixed.
	cc.	*mg.*	*mg.*
1	0.20	0.61	.....
2	.75	2.28	1.67
3	1.25	3.80	3.19
4	2.80	8.51	7.90

These tabulations show that the fixation of nitrogen by *A. vinelandii* increased with the surface exposure. This circumstance is clearly due to two reasons. In the first place the bacteria of the azotobacter group are aerobic organisms, and with them the fixation of nitrogen takes place where the access of oxygen is greatest; that is, at the surface. Where the surface exposure is greatest there also growth is greatest, and where growth is more vigorous the building of the organic nitrogenous substance in the bacterial bodies is also most active. In the second place, the larger surface exposed places at the disposal of the bacteria not only a greater amount of oxygen but also a greater amount of gaseous nitrogen, and this is utilized readily when the bacterial growth is vigorous.

SERIES VIII.

In growing *A. vinelandii* in mannite solutions in which strips of filter paper had been placed, it was noticed that in the presence of the latter growth was far more abundant than in the tubes where no paper was present. The question naturally arose whether *A. vinelandii* is capable of utilizing part of the filter paper as a source of carbon and whether by its aid it is capable of fixing greater quantities of nitrogen. To decide this point the following experiment was arranged:

1. 150 cc mannite solution + 1 gram calcium carbonate.
2. 150 cc mannite solution + 2 grams filter paper.
3. 150 cc mannite solution.
4. 150 cc mannite solution + 2 grams filter paper.

The mannite solution used contained 5 mg of potassium nitrate per liter and the flasks were the ordinary Jena, round-bottomed flasks, used for the Kjeldahl digestion. At the end of the experiment it was merely necessary to take out the cotton stopper, add mercury and sulphuric acid, and proceed with the work as in other Kjeldahl determinations. In this way it became unnecessary to transfer the culture solutions at the end of the experiment, and thus a possible error was avoided. After sterilization in the autoclave and cooling, 3 and 4 were inoculated from a pure culture of *A. vinelandii*, while 1 and 2 were allowed to remain sterile. In 48 hours the several flasks appeared as follows:

1. Clear.
2. Clear.
3. Cloudy, floating membrane.
4. Turbid, floating membrane.

In three weeks the solutions in this series were analyzed and the following amounts of nitrogen were found:

No.	Ammonium hydrate (0.003012 gram nitrogen per cubic centimeter).	Nitrogen found.	Nitrogen fixed.
	cc.	*mg.*	*mg.*
1	0.15	0.45	.....
2	.65	1.96	.....
3	4.10	12.35	11.90
4	9.85	29.67	27.71

It will be seen from the above that the sterile filter paper contained 1.96 mg less 0.45 mg, or 1.51 mg of nitrogen. Comparing the amounts of nitrogen fixed in 3 and 4, we find that in the presence of the filter paper there was fixed in 4 more than twice as much nitrogen as was fixed in 3. The 150 cc of the mannite solution contained 3 grams of mannite, hence there were fixed in 3, 3.97 mg of nitrogen for every gram of mannite consumed. Disregarding for the moment the filter paper as a source of organic carbon, we find that there were fixed in 4, 27.71 mg of nitrogen for the 3 grams of mannite consumed, or nearly 9.24 mg of nitrogen for every gram of mannite used. It is pertinent to ask here whether part of the filter paper was hydrolyzed and then used by the bacteria as a source of food. Another series of experiments reported below seems to indicate that the filter paper is not used by the bacteria as a source of carbon. It does not follow, however, that such utilization of the cellulose in the soil humus does not take place.

Series VIII a.

The arrangement of this series was as follows:

1 and 2. 200 cc mannite solution+1 gram filter paper, sterile.
3 and 4. 200 cc mannite solution+1 gram filter paper, *A. vinelandii.*

At the end of four weeks the cultures were sterilized, the filter paper was removed after careful rinsing, and the residues analyzed. The following amounts of nitrogen were found:

No.	Ammonium hydrate (0.001307 gram nitrogen per cubic centimeter).	Nitrogen found.	Nitrogen fixed.
	cc.	*mg.*	*mg.*
1	0.25	0.32	.....
2	.20	.26	.....
3	9.00	11.76	11.44
4	8.75	11.44	11.12

The filter paper when dried and weighed showed no appreciable change in weight. Unintentionally the parallel cultures containing no filter paper were left out, so that the results here can not be claimed to prove with certainty that the filter paper is not attacked by a vigorously growing culture of *A. vinelandii;* nevertheless, it would appear from this that where the presence of the filter paper encourages growth it is not necessarily due to the combined carbon offered by the paper to the bacteria.

SERIES IX.

In this series, as in the preceding, filter paper was used together with mannite. The different proportions of the paper used were intended to show whether there is a point beyond which the increase in the quantity of paper produces no corresponding increase in the amount of nitrogen fixed. The arrangement of the experiment was as follows:

(1) 200 cc mannite solution.
(2) 200 cc mannite solution +0.5 gram paper.
(3) 200 cc mannite solution +1.0 gram paper.
} Sterile.
(4) 200 cc mannite solution.
(5) 200 cc mannite solution +0.5 gram paper.
(6) 200 cc mannite solution +1.0 gram paper.
(7) 200 cc mannite solution +2.0 gram paper.

There was also added to each flask 1 gram of calcium carbonate. In four days the several solutions appeared as follows:

(1) Clear.
(2) Clear.
(3) Clear.
(4) Turbid, pigment, surface film.
(5) Turbid, pigment, surface film.
(6) Turbid, pigment, surface film.
(7) Turbid, pigment, surface film.

At the end of 10 days the contents of the flasks were analyzed and the following amounts of nitrogen found:

No.	Ammonium hydrate (0.00302 gram N. per cubic centimeter).	Nitrogen found.	Nitrogen fixed.
	cc.	*mg.*	*mg.*
1	0.15	0.45	.....
2	.30	.91	.....
3	.40	1.21	.....
4	2.30	6.95	6.50
5	3.60	10.87	9.96
6	3.80	11.48	10.27
7	3.75	11.32	9.81

The mannite solution used here was like that used in the other series, except that it contained 0.15 gram of mannite per liter, instead of 0.20 gram; the 200 cc of mannite solution used here contained in each case 3 grams of mannite. With these 3 grams of organic substance there were fixed in 4, 6.40 mg of nitrogen, or 2.17 mg of nitrogen for every gram of mannite used up. In 5, where there was 0.5 gram of filter paper added to the 250 cc of solution, there was a fixation of 9.96 mg, or of 3.32 mg for every gram of mannite used up. When the quantity of paper was still further increased in 6, the amount of nitrogen fixed also increased, and we find here a fixation of 10.27 mg, or of 3.42 mg of nitrogen per gram of mannite employed. A still further increase in the quantity of filter paper used not only failed to yield an increased amount of fixed nitrogen, but actually led to a diminution in the amount of nitrogen fixed. Thus we find in 7 a total fixation of 9.81 mg of nitrogen, or of 3.27 mg per gram of sugar consumed. This is slightly less than the amount fixed in 5, where only 0.5 gram of filter was used. This experiment confirms the results obtained in Series VII, but it also shows that there is a maximum quantity of filter paper that can be advantageously used. Evidently the presence, in 200 cc of the

mannite solution, of 2 grams of filter paper was less favorable for the fixation of a maximum amount of nitrogen than the presence in the same solution of only 1 gram of paper. Larger proportions than 2 grams of paper to 200 cc of solution were not tried. It is probable, however, that a further increase in the amount of paper used would still further diminish the fixation of free nitrogen. Now, since cellulose is rather insoluble in water, it seems strange that the presence of a mass of inert material should unfavorably influence the development of *A. vinelandii*. There is a possibility, however, that the depressing effect of the larger quantities of cellulose are due to the combined nitrogen contained in it. Reference has already been made to Winogradsky's experiment, where his *Clostridium pasteurianum* actually caused a loss of nitrogen from the solution when the amount of combined nitrogen added exceeded a certain proportion of the amount of sugar in solution. Also Beijerinck and Van Delden and Gerlach and Vogel have observed that larger quantities of combined nitrogen discourage the fixation of free nitrogen by bacteria of the azotobacter group. It will be seen that No. 5 contained 0.46 mg of combined nitrogen in the filter paper, No. 6 contained 0.76 mg, and No. 7 1.51 mg of combined nitrogen.

Series X.

The arrangement of the cultures in this series was as follows:

(1) 100 cc mineral solution+1 gram filter paper.
(2) 100 cc mineral solution+1 gram filter paper +0.1 gram mannite.
(3) 100 cc mineral solution+1 gram filter paper +0.2 gram mannite.
(4) 100 cc mineral solution+1 gram filter paper +0.5 gram mannite.
(5) 100 cc mineral solution+1 gram filter paper +1.0 gram mannite.
(6) 100 cc mineral solution+1 gram filter paper +1.5 gram mannite.

The several solutions were inoculated with *A. vinelandii* and kept in the incubator for 12 days. At the end of that time the cultures were analysed and the following amounts of nitrogen were found:

No.	Ammonium hydrate (0.00302 gram N. per cubic centimeter).	Nitrogen found.	Nitrogen fixed.
	cc.	*mg.*	*mg.*
1	0.40	1.21	.....
2	.75	2.26	1.05
3	.95	2.87	1.66
4	1.40	4.23	3.02
5	1.95	5.89	4.68
6	2.00	6.04	4.83

The greatest proportionate fixation took place in Nos. 2 and 4, where 1 gram and 5 grams, respectively, of mannite per liter were used. This would mean, in the latter case, a fixation of 6.04 mg of nitrogen per gram of mannite consumed. As the amount of mannite increased from 0.5 gram to 1 gram—that is, from 5 to 10 grams per liter—the yield of combined nitrogen also increased, but not correspondingly. While the increase of mannite was 100 per cent, the increase in the amount of nitrogen fixed was only 53 per cent. A further increase of the amount of mannite in solution—namely, to 15 grams per liter—was followed by a further, but not proportionate increase in the amount of nitrogen fixed. In fact, the increase here was but slight. Evidently the increasing amount of organic substance finally begins to retard the fixation of gaseous nitrogen and, as was noted in Series IX, there is a limit not only for the soluble nonnitrogenous organic material, but also for the insoluble nitrogen-poor substance, as represented here by the cellulose of the filter paper.

SYMBIOTIC FIXATION BY A. VINELANDII AND B. 30.

The fixation of nitrogen by *A. vinelandii* was stimulated by the presence in the culture solution of a small bacillus described elsewhere,[a] and designated by the laboratory number *B. 30*. Nitrogen determinations showed that this organism can fix for itself only slight quantities of free nitrogen, and it was naturally of great interest to decide just to what extent the latter contributed to the fixation of atmospheric nitrogen when in symbiotic growth with *A. vinelandii*. A number of experiments were carried out, which show very definitely that in the presence of *B. 30*, *A. vinelandii* is capable of fixing much larger quantities of nitrogen than when growing alone.

SERIES XIV.

This series was arranged to complete Series X, and is presented here accordingly. The arrangement was as follows:

(1) 100 cc mineral solution+1 gram filter paper.
(2) 100 cc mineral solution+1 gram filter paper +0.1 gram mannite.
(3) 100 cc mineral solution+1 gram filter paper +0.2 gram mannite.
(4) 100 cc mineral solution+1 gram filter paper +0.5 gram mannite.
(5) 100 cc mineral solution+1 gram filter paper +1.0 gram mannite.
(6) 100 cc mineral solution+1 gram filter paper +1.5 gram mannite.
(7) 100 cc mineral solution+1 gram filter paper.
(8) 100 cc mineral solution+1 gram filter paper +0.1 gram mannite.
(9) 100 cc mineral solution+1 gram filter paper +0.2 gram mannite.
(10) 100 cc mineral solution+1 gram filter paper +0.5 gram mannite.
(11) 100 cc mineral solution+1 gram filter paper +1.0 gram mannite.
(12) 100 cc mineral solution+1 gram filter paper +1.5 gram mannite.

Nos. 1 to 6, inclusive, were inoculated with *A. vinelandii;* Nos. 7 to 12, inclusive, were inoculated with *A. vinelandii* + *B. 30*. The cultures were kept in the incubator at 28° C., and were analyzed at the end of twelve days. The following amounts of nitrogen were found:

No.	Ammonium hydrate (0.00302 gram N. per cubic centimeter).	Nitrogen found.	Nitrogen fixed.
	cc.	*mg.*	*mg.*
1	0.40	1.21	.....
2	.75	2.26	1.05
3	.95	2.87	1.66
4	1.40	4.23	3.02
5	1.95	5.89	4.68
6	2.00	6.04	4.83
7	.40	1.21	.....
8	.90	2.72	1.51
9	1.30	3.93	2.72
10	2.40	7.25	6.04
11	3.25	9.81	8.60
12	3.20	9.67	8.46

The results obtained in solutions Nos. 1 to 6 were discussed under Series X. It remains now to compare the second half of this series with the first half of it. It will be seen that in every case where *B. 30* was inoculated together with *A. vinelandii* there was increase in the amount fixed over and above that yielded by *A. vinelandii* alone. Culture solution No. 7 should be excepted, of course, for no appreciable fixation of nitrogen took place in that case. In order to bring out these relations still more clearly, the corresponding yields are arranged parallel to one another.

[a] J. G. Lipman, Nitrogen Fixing Bacteria, Doctor's Thesis, Cornell University, June, 1903.

No.	Fixation by *A. vinelandii.*	No.	Fixation by *A. vinelandii*+*B. 30.*	Increase.
	mg.		*mg.*	*Per cent.*
2	1.05	8	1.51	43.8
3	1.66	9	2.72	63.8
4	3.02	10	6.04	100.0
5	4.68	11	8.60	83.7
6	4.83	12	8.46	75.1

The greatest proportionate fixation was in Nos. 2 and 8, respectively. As the amount of mannite increased, the proportionate fixation decreased. There were fixed, per gram of mannite consumed, the following quantities of nitrogen:

	Mg.		*Mg.*
No. 2	10.50	No. 8	15.10
No. 3	8.30	No. 9	13.60
No. 4	6.04	No 10	12.08
No. 5	4.68	No. 11	8.60
No. 6	3.22	No. 12	5.64

It is but natural that with a smaller quantity of food in solution the bacteria should utilize it more thoroughly, and thus reduce the waste to a minimum. The experiment of Gerlach and Vogel, cited above, confirms the results presented here. There is a very close analogy in this case to the utilization of combined nitrogen in the soil by higher plants. The greater the amount of it there is present in the soil, the slighter the proportionate utilization of it within given limits. In this case the presence of *B. 30* enabled a more economical use of the organic substance in solution; nevertheless, even with the two organisms working together, there was a gradual decrease in the proportionate amount of nitrogen fixed. On the average, the presence of *B. 30* enabled *A. vinelandii* to fix 73.3 per cent more nitrogen. The minimum is 43.8 per cent, in No. 8, and the maximum 100 per cent, in No. 10.

Repeated experiments which it would be superfluous to give here in detail still further emphasized the facts noted above. They all show that aside from the increased fixation, the presence of *B. 30* also enables *A. vinelandii* to withstand better the presence of excessive quantities of organic matter. Hence, it would appear that the accumulation of large quantities of organic matter in the soil, be it rich or poor in nitrogen, unfavorably affects the development of azotobacter bacteria. When there is an accumulation of large quantities of nitrogen-rich substance, the growth of these organisms practically stops, when there is an accumulation of nitrogen-poor organic substance, the growth of these organisms is retarded. But since in all arable soils many bacterial species act and interact on one another, and the development of any particular combination may become prominent at one time and of another at some other time, it is clear that the conditions of fixation in the soil are perforce somewhat different from those existing in artificial cultures. None the less, they show unequivocally that, for its best work, *A. vinelandii*, and undoubtedly also other members of the azotobacter group, must depend on the cooperation of other organisms.

Series XVI.

This series is complimentary to Series XVII, and was carried out with a pure culture of *A. vinelandii*. It was intended to study the effect on fixation of a large exposed surface. For this reason the mannite solution employed was placed in large crystallizing dishes covered in the manner of ordinary Petri dishes. The arrangement in this series was as follows:

(1) Large Erlenmyer flask, 300 cc mannite solution, sterile.
(2) Large Petri dish, 300 cc mannite solution, *A. vinelandii.*
(3) Large Petri dish, 500 cc mannite solution, *A. vinelandii.*

The internal diameter of the dish in No. 2 was 27 cm. and in No. 3, 29 cm. The mannite solution contained 15 grams of mannite per liter; hence Nos. 1 and 2 contained 4.5 grams of mannite each, and No. 3 contained 7.5 grams of mannite. After sterilization, cooling, and inoculation in the usual manner, the dishes were placed in the incubator at a temperature of 28° C. In both of the inoculated dishes growth was apparent in twenty-four hours. In forty-eight hours there was the development of the bright yellow pigment observed in all mannite cultures of *A. vinelandii*, and of the characteristic membrane on the surface. As it grew older the membrane became gradually thicker. On examination under the microscope it was found that *A. vinelandii* alone was present, and hence there had been no contamination from the outside. The contents of the dishes and of the flask were analyzed at the end of six days, and the following amounts of nitrogen were found:

No.	Ammonium hydrate (0.00302 gram N. per cubic centimeter).	Nitrogen found.	Nitrogen fixed.
	cc.	*mg.*	*mg.*
1	0.20	0.60	.....
2	9.55	28.84	28.24
3	17.40	52.55	51.95

During digestion part of the solution in No. 2 was lost, and the amount given here is undoubtedly somewhat too low. While the loss is not believed to have been very considerable, it amounted to 2 or 3 mg. Leaving No. 2 out of consideration, we note that there were 51.95 mg of nitrogen fixed in No. 3; hence there was a fixation of 6.8 mg of nitrogen for every gram of mannite consumed. This is a very considerable quantity for a period of six days, and by a pure culture of *A. vinelandii*. These results were obtained with a culture which had been reinoculated into artificial solutions a considerable number of times, and they show that *A. vinelandii* does not lose its nitrogen-fixing power, as was reported in the case of other nitrogen-fixing organisms.

Series XVII.

The culture solutions in this series were arranged as follows:

(1) 200 cc mannite solution in Erlenmeyer flask + 1 gram filter paper.
(2) 200 cc mannite solution in large Petri dish + 1 gram filter paper.
(3) 200 cc mannite solution in large Petri dish + 1 gram filter paper.

No. 1 was left sterile, No. 2 was inoculated with *A. vinelandii*, and No. 3 with *A. vinelandii* + *B. 30*. There was also added in each case 1 gram of calcium carbonate.

At the end of 10 days the several solutions were analyzed and the following amounts of nitrogen were found:

No.	Ammonium hydrate (0.00302 gram N. per cubic centimeter).	Nitrogen found.	Nitrogen fixed.
	cc.	*mg.*	*mg.*
1	0.15	0.45	.....
2	9.90	29.90	29.45
3	10.15	30.65	30.20

The solutions contained at the beginning of the experiment 3.0 grams of mannite in each case, so that there were fixed in No. 2, 9.81 mg of nitrogen for every gram of

mannite consumed. Similarly, there were fixed in No. 3, 10.07 mg of nitrogen for every gram of mannite consumed. These are the highest yields thus far obtained in the 15 grams of mannite per liter. The presence of *B. 30* increased the yield also in this case, although the increase was rather small.

FIXATION OF NITROGEN BY A. VINELANDII IN THE PRESENCE OF OTHER SOIL BACTERIA.

These studies include experiments with a number of organisms. Their purpose was to determine whether there are bacteria other than *B. 30* that would favor the fixation of free nitrogen by *A. vinelandii*.

SERIES XVIII.

The solutions employed in this series contained, besides the customary amounts of the mineral salts, either 20 grams of mannite, or of glucose, per liter of solution. Two hundred cc portions of these solutions were distributed in Kjeldahl flasks, with the addition in each case of 1 gram of calcium carbonate. The organisms used in this series were *A. vinelandii*, *B. pyocyaneus*, *B. 31*, *B. 30*, *B. 31* var., and *B. 32*. The arrangement of the experiment was as follows:

(1) Glucose solution. } Sterile.
(2) Mannite solution. }
(3) Glucose solution. } *A. vinelandii.*
(4) Mannite solution. }
(5) Glucose solution. } *A. vinelandii* + *B. pyocyaneus.*
(6) Mannite solution. }
(7) Glucose solution. } *A. vinelandii* + *B. 31.*
(8) Mannite solution. }
(9) Glucose solution. } *A. vinelandii* + *B. 30.*
(10) Mannite solution. }
(11) Glucose solution. } *A. vinelandii* + *B. 31* var.
(12) Mannite solution. }
(13) Glucose solution. } *A. vinelandii* + *B. 32.*
(14) Mannite solution. }

At the end of 8 days the cultures were analyzed and the following amounts of nitrogen found:

No.	Ammonium hydrate (0.003038 gram N. per cubic centimeter).	Nitrogen found.	Nitrogen fixed.
	cc.	*mg.*	*mg.*
1	0.25	0.76	.....
2	0.15	0.45	.....
3	1.35	4.10	3.34
4	1.45	4.40	3.95
5	1.55	4.71	3.95
6	0.90	2.73	2.28
7	1.55	4.71	3.95
8	1.55	4.71	4.26
9	3.25	9.87	9.11
10	3.55	10.78	10.33
11	1.55	4.71	3.95
12	1.85	5.62	5.17
13	1.35	4.10	3.34
14	1.95	5.92	5.47

These results show that, with one exception, there was more nitrogen fixed in the mannite solutions than there was in the glucose solutions. They also show that

B. 30 was the only organism of those tried in the series which increased the fixation of nitrogen by *A. vinelandii* to any considerable extent. Thus *A. vinelandii* alone fixed in glucose 3.34 mg of nitrogen, and in mannite 3.95 mg of nitrogen. In the presence of *B. 30* the corresponding amounts were 9.11 mg and 10.33 mg—more than 2.5 times as much. It also seems that in Nos. 12 and 14 there was a marked increase in the amount of nitrogen fixed, due to the presence in No. 12 of *B. 31* var., and in No. 14 of *B. 32*. Further combinations of *A. vinelandii* with one or more of the other organisms showed that none of them gave any considerable increase beyond that given by *B. 30*.

The experiments presented here are sufficient to show to what extent the members of the azotobacter group are capable of fixing atmospheric nitrogen in artificial solutions. There are many other experiments at hand representing hundreds of analyses which are confirmatory of the above, and it is now proposed to extend the experimental work to actual soil conditions, in order to discover whether the bacteria of this group may be utilized to replace the nitrogen removed from the soil by crops.

Mr. Withers. I have a resolution which I shall be glad to have referred to the committee on recommendations of the referee on soils. It is as follows:

Resolved, That the referee on soils be requested to test if possible the value of proposed methods for comparing the ability of different soils to support the growth of nitrifying organisms when the conditions are identical as to temperature, moisture, number of germs, etc.

The President. If there is no objection this resolution will be referred to the committee on recommendations on soils.

Mr. Wheeler. I have no formal paper, Mr. President, but I wish to call attention to a new indictment that we have to bring against ignited iron and aluminum phosphate or roasted "redondite." This redondite contains from 35 to 40 per cent of reverted phosphoric acid and hence is a convenient material with which to raise the percentage of "available" phosphoric acid in mixed fertilizers. Persons engaged in the fertilizer business say that it is used extensively, and yet this phosphate when employed on acid soil has very little value, and as concerns its after effect is practically worthless. This is a very serious indictment to bring against a material which contains from 35 to 40 per cent of available or reverted phosphoric acid. Phosphate, however, when applied upon a limed soil is much more valuable. The limed soil used was probably not limed heavily enough to bring all of the organic acids into union with lime, though it may have been temporarily neutral or alkaline to litmus paper. It was sufficiently limed, however, to produce all ordinary crops satisfactorily.

Upon this limed soil and with certain crops this phosphate was nearly equal, in the results produced, to some of the most valuable phosphates. This was true of grass for a period of about four years. But when used with many of the hoed crops, particularly with turnips, beets, squashes, lettuce, etc., this material was of small value, even upon the limed soil, as compared with basic slag-meal and finely

ground bone, either acidulated or unacidulated, and with other phosphates. In Rhode Island we are face to face with this proposition—either our soils must be put in a condition such that this material will be effective or our method of analysis must be modified so that we may discriminate against this particular phosphate. As the chemists can not compel farmers to lime their acid soils it seems that we should modify our methods or possibly determine the alumina in the goods as an indication of the presence of this phosphate. The leading facts concerning this matter as ascertained to date may be summarized as follows:

Panicum crus-galli, common millet, and a few other plants, seem to have exceptional ability to use ignited aluminum phosphate on both limed and unlimed soil. Its efficiency for these crops is increased, nevertheless, by liming. For grass on limed soil, its efficiency was very close to that of some of the best phosphates, but upon acid soil its relative and absolute efficiency were much less. For many of the hoed crops, Swedish turnips, beets, flat turnips, squashes, lettuce, and others, its after effect, at least upon acid soil, amounts to practically nothing. Its after effect upon limed soil is relatively better than upon the unlimed soil, yet the crops, even then, were poor compared with those produced by the lime phosphates, acidulated or unacidulated. Those plants which seem to make the best use of ignited aluminum phosphate on unlimed soil are generally those least affected by acidity. This may be merely because they are able to exist under such acid conditions rather than on account of any special ability to feed upon this particular phosphate.

This is a brief summary of the conclusions reached and in view of these facts I wish to bring before the association as forcibly as possible the desirability of taking under immediate consideration our methods for determining reverted phosphoric acid in commercial fertilizers. This particular material is introduced very largely into our commercial fertilizers not only by those who control the process of making it, but also by others, and one of the principal reasons for its employment is that it produces a fertilizer which drills readily, a feature that pleases the farmer. I wish that some member of this association who lives on an alkaline soil would test this same material for a series of years and find out if under such soil conditions this phosphate is as effective as the other phosphates even for such crops as turnips and beets.

Mr. McDonnell. It is interesting to note in connection with Mr. Wheeler's remarks that the fact that the aluminum phosphates are more available in alkaline soils agrees with the fact that these phosphates are more soluble in the citrate solution when it is slightly alkaline than when it is slightly acid.

Mr. Wheeler. Referring to some remarks previously made I wish to give a caution against using enough lime to bring all the organic acids into combination at the outset, because I believe that practically all the benefit to be derived from liming may be secured at once by using less than that amount even on very acid soils. At least for a few years the use of much less lime than is necessary to permanently

neutralize the total acidity of the soil will insure good crops and the agricultural chemist must consider the economical aspect of such matters. While it might be well to neutralize all the acidity at once, or, in other words, combine if possible all of the organic acids and other acid substances with lime, it would be quite expensive, and hence it is better to effect this only partially at first and later to neutralize completely.

At 4.20 p. m. the association adjourned.

THIRD DAY.

SATURDAY—MORNING SESSION.

The convention was called to order by the president at 9.30 a. m. The report on phosphoric acid was called for and in the absence of the referee, who had sent no formal report but in a letter to the secretary had made certain observations relative to the work, the following informal report was submitted by the secretary:

REPORT ON PHOSPHORIC ACID.

By B. H. Hite, *Referee.*

(1) The association made no recommendations or suggestions in regard to the determination of phosphoric acid, which the referee thinks was wise. The present method for determining phosphoric acid meets every requirement, while the shortcomings in the methods for the determination of other constituents could well consume all the time at the disposal of the association for cooperative work.

(2) The associate referee was anxious to test yet other modifications of the volumetric method as applied to the determination of phosphoric acid in soils. The referee did not care to take part in any further cooperative work on this subject, and so instructed the associate to take full charge of that investigation, sending out his own samples, etc.

(3) The referee was anxious to do something toward improving existing methods and turned his attention to certain modifications of well-known methods involving the weighing of the yellow precipitate. In the hands of the referee the methods gave most excellent results, but when tried by other analysts indifferent results were obtained. The work was again taken up by the referee, and it is believed that the cause of the varying results can be located, but it is not deemed advisable to present the work to the association until more time has been given to it.

Mr. Van Slyke. In this connection I wish to submit by title a paper on "The Determination of Organic and Inorganic Phosphorus in Vegetable and Animal Materials," by E. B. Hart and W. H. Andrews, of the Geneva station.[a]

Mr. Williams. I have a report on phosphoric acid to submit to the association.

COMPARISON OF THE VOLUMETRIC AND GRAVIMETRIC METHODS IN DETERMINING TOTAL PHOSPHORIC ACID IN SOILS.

By C. B. Williams, *Associate referee.*

The work of the associate referee on phosphoric acid was confined this year entirely in testing the relative efficiency of the volumetric and gravimetric methods

[a] This paper may be found in Bulletin No. 238 of the New York (Geneva) station, and in the American Chemical Journal, vol. 30, No. 6, December, 1903.

in the determination of total phosphoric acid in four soils, two from North Carolina and one each from California and Illinois. This was undertaken because it is believed that a knowledge of the total quantity of plant food in any soil will afford a more complete understanding of its potentialities, cultural, and fertilizer requirements than results obtained by any other method. A circular letter was sent to the chief chemists of all agricultural experiment stations asking cooperation in the work for this year. Samples were sent to the seven who replied favorably, but analytical data have been supplied only by three analysts.

Directions for Work on Phosphoric Acid Samples for 1903.

I.—MOISTURE.

Use the official method described in Bulletin 46, revised edition, Bureau of Chemistry, U. S. Department of Agriculture, page 71.

II.—METHOD OF SOLUTION.

Place 3 grams of soil in a platinum dish and ignite until organic matter has been destroyed, then treat three times with from 3 to 4 cc of hydrofluoric acid, evaporating to dryness each time on a water bath, using a platinum rod to stir upon each addition of acid. Mix the residue thus obtained with 10 grams of a mixture of equal parts of sodium and potassium carbonates (free from phosphoric acid), and reduce in an agate mortar to a fine powder, after which heat over a blast lamp, gently at first, until the mass has completely agglutinated, when stronger heat should be turned on and continued until calm fusion is attained. Then cool, place the dish and its contents in a beaker and add sufficient (1:1) hydrochloric acid to cover the dish. Place on a water bath and digest until the mass has thoroughly loosened from the dish, after which it is removed. Evaporate to dryness on a water bath and thoroughly dehydrate the silica present by finishing the heating in an air bath at 110° C. for 4 hours. Take up with dilute hydrochloric acid and digest on water bath for 30 minutes, after which filter from silica, and wash thoroughly to remove traces of phosphoric acid. To the filtrate add sufficient nitric acid to liberate all chlorids and evaporate the solution to a volume of about 40 cc. Then neutralize the excess of nitric acid with ammonia and add 12 grams of ammonium nitrate.

After effecting solution as indicated above, determine phosphoric acid in the four soil samples by the following methods:

III.—GRAVIMETRIC METHOD.

The regular official method (Bul. 46, revised edition, Bureau of Chemistry, U. S. Department of Agriculture, p. 74).

IV.—VOLUMETRIC METHOD.

Cool, add 30 cc of recently filtered molybdic solution, and securely stopper (antimony rubber stoppers have been found most satisfactory). Shake the flask in a shaking machine or by hand for 30 minutes, after which remove and filter through carbon filters by means of suction, using asbestos mats.

After thoroughly transferring the ammonium phospho-molybdate precipitates and washing out the flasks onto the asbestos filters, give the precipitates six more washings each. Then remove the stoppers from the pressure flasks with the small ends of the carbon filters still stuck through them and hold upright over the sink, washing the outsides free from acid with distilled water. Reverse the carbon filters into the mouths of the flasks which originally contained the precipitates, still holding the small stems, and by means of the copper wires that extend beyond the small ends of the carbon filters push out the disks, asbestos, and precipitates into the flasks and wash the disks and inside of carbon filters carefully with a small stream of water. Dissolve the precipitates in a slight excess of standard alkali, add a few drops of phenolphthalein solution to each and titrate back with standard (nitric) acid, using a long stirring rod to thoroughly agitate during the operation.[a]

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[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. No. 46, pp. 13–14; J. Amer. Chem. Soc. **23**: 8.

(*a*) *Apparatus.*

1. *Precipitating flask.*—This consists of a 500 cc Erlenmeyer flask with neck 40 mm inside diameter and rubber stopper to fit.

2. *Filtering device.*—Through a rubber stopper in a 16 ounce pressure bottle of Erlenmeyer form is passed the small end of a carbon filter; in the bottom of this is placed a perforated porcelain plate or disk, to which is rigidly fastened a No. 19 copper wire about 25 cm long that projects downward into the pressure bottle. The disk is covered with a thin layer of asbestos prepared according to Gooch.

3. *Shaking machine.*—Use the apparatus devised by Wagner, except that the caps that fit over the tops of the flasks are made larger. This machine holds ten flasks and can be revolved either by hand or motor. A speed of 40 to 50 revolutions per minute should be maintained, as this is the velocity that has been found to give the maximum agitating efficiency.

(*b*) *Preparation of reagents.*

The reagents are as indicated in the official methods, except that the strength of the acid and alkali is one-half the strength there prescribed; i. e., one cc will be equal to one-half milligram phosphoric acid (0.01 per cent phosphoric acid on a basis of 5 grams of soil).

1. *Distilled water.*—All washing and diluting should be with distilled water. Distilled water obtained from water containing a large amount of bicarbonates is liable to contain considerable carbon dioxid in solution. This water can be used in washing the ammonium phosphomolybdate, but should be used neither in transferring the precipitate nor in diluting before titration. Boiling the water prior to using will obviate this difficulty.

V. REPORTING RESULTS.

All results should be tabulated, after calculating to water-free basis, and sent with complete notes to the associate referee at least thirty days prior to the meeting.

CHARLES B. WILLIAMS,
Associate Referee.

DESCRIPTION OF SOILS.

No. 1.—This soil is a mellow, fine, sandy loam of sedimentary origin, underlaid by a moderately retentive clay subsoil at a depth of 7 inches. It was taken from the middle of the fertilizer test plats of corn in center of third series of unfertilized plats at the Edgecombe test farm of the North Carolina department of agriculture. This soil represents a very extensive type in the eastern part of the State that is especially adapted to the production of cotton, corn, peanuts, truck, and bright-leaf tobacco. This land has been in cultivation for about 75 years and has been principally devoted to cotton, corn, oats, wheat, tobacco, and clover. Its original forest growth was pine, oak, and sweet and black gum.

No. 2.—Heavy red clay loam, designated by the Bureau of Soils as Cecil clay, was taken at a depth of 8 inches from plat No. 5, second series of unfertilized corn plats of the Statesville test farm of the North Carolina department of agriculture. This soil is of residual origin, "formed in situ, by the long continued processes of decomposition, from a number of rock types distinct in their physical and mineralogical characters. The soil has been derived from nearly all of the rocks occurring in this section, mainly hornblende gneiss, micaceous schists, coarse-grained granites, and, to a less extent, soapstone or steatite."

Although the soil is underlaid by a stiff tenacious red clay subsoil to a depth of several feet, it possesses good natural drainage. It is a strong, valuable soil and is especially adapted to the growing of grains, grasses, and clover. Cotton will grow upon it, but it is not so well suited to this crop as to the ones first mentioned. The original native growth was principally black-jack oak, pine, and black and sweet gum.

No. 3.—This is a dark soil, containing considerable humus to the depth of 7 inches, taken from the terrace or second bottom land along the Du Page River in Du Page County, Ill. It lies from 20 to 30 feet above high-water mark and probably has not been overflowed for centuries, although it may have at one time been bottom land of the Du Page River. It is made up largely of glacial drift, somewhat modified by the underlying limestone deposits. It belongs to one of the richest classes of agricultural soils of the State of Illinois. The land has been in alfalfa for three years and has produced as high as 10½ tons of alfalfa hay per acre in a single season. Under favorable seasonal conditions, says Mr. Cyril G. Hopkins, who furnished the soil, it will undoubtedly produce from 90 to 100 bushels of corn per acre.

No. 4.—This sample, furnished by Director E. W. Hilgard, is a fine, heavy, dark clay soil, collected from near the town of Arroyo Grande, in the valley of Arroyo Grande, in the southern portion of San Luis Obispo County, Cal. This soil is very rich in plant food and is especially noted among seedsmen as one "capable of producing vegetable seeds of maximum value." Over one-half of this soil is silt, and possesses a saturation point by volume of 67 per cent and by weight of 77 per cent. The mechanical and chemical analyses of this soil (No. 2061) will be found on page 178 of the Report of the California Agricultural Experiment Station for the years ending 1898–1901, Part II.

Results of Work.

The analytical results, given in the table, are calculated to water-free substance, and hence are on a strictly comparable basis.

Determination of total phosphoric acid by two methods in four (calculated to water-free substance) soils.

Analyst.	No. 1.		No. 2.		No. 3.		No. 4.	
	Volumetric.	Gravimetric.	Volumetric.	Gravimetric.	Volumetric.	Gravimetric.	Volumetric.	Gravimetric.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
F. P. Veitch, Washington, D. C	0.043	0.037	0.086	0.077	0.272	0.322	0.468	0.529
W. G. Haywood, Raleigh, N. C	.....	.....	.....	.....	.289	.....	.520	.....
	.....	.....	.....	.....	.308	.311	.499	.517
C. B. Williams, Raleigh, N. C	.....	.093	.....	.079	.305	.336	.501	.496
	.044	.096	.076	.080	.302	.296	.492	.503
J. H. Pettit, Urbana, Ill.[a]	.050	.048	.049	.049	.276	.274	.495	.500
Average	.043	.066	.081	.079	.291	.316	.492	.515

[a] Received too late to be included in averages; see comments by analyst.

Comments by Analysts.

F. P. Veitch.—The instructions were followed in all essential particulars. In the execution of the volumetric method the yellow precipitate was filtered and washed free of acid on the ordinary filter paper and funnel. No shaker was used, but the precipitate was allowed to stand four hours at 50° C. before filtering. In regular work with the gravimetric method it is my practice, after dissolving in ammonia, to reprecipitate with nitric acid and more molybdate and complete the determination as usual, washing the precipitate very thoroughly with water. In routine work we always use the volumetric method on soils.

Harry Snyder.—One set of determinations was made on the soil samples, but for lack of time have not been able to duplicate the results. Mr. Hummel, the assistant chemist, who did the work, reports that the results by the gravimetric method are slightly higher than those obtained by the volumetric. In some soil work we have

divided the solution, used part for the gravimetric and part for the volumetric determination, and then dissolved the gravimetric precipitate after weighing, and determined the phosphoric acid volumetrically. Determinations made in this way compare quite closely. I think the difficulty is due to the presence of a little alumina and other compounds in the final precipitate, and on that account the results by the volumetric method are more satisfactory than those by the ordinary gravimetric process.

W. G. Haywood.—The results on soils Nos. 1 and 2 were not altogether satisfactory, and no report is made on them, as sufficient time was not available to make further determinations. Considerable difficulty was experienced with Nos. 1 and 2 in keeping the phosphoric acid from going out of solution while being evaporated with nitric acid prior to adding molybdic solution; with soils Nos. 3 and 4 no trouble was encountered. Of the two methods, I prefer the volumetric, as it is easier to manipulate than the gravimetric and gives equally as good results.

J. H. Pettit.—I had difficulty in running the method suggested for getting the phosphoric acid into solution. In our regular work on soils we use the 10-hour digestion method for obtaining the soil solution. For the determination of phosphoric acid in this solution an aliquot portion is concentrated to 25 cc, neutralized with ammonia and cleared up with nitric acid. Then heat to 40°, precipitate with molybdic solution and let stand over night. Filter, wash, dissolve in standard potassium hydroxid, and titrate the excess of the latter with standard nitric acid. This is practically the same method as that outlined by the association and has been checked up with the gravimetric method. Further, for my personal satisfaction, I used the 10-hour digestion on the soils sent out by the referee, intending to compare the results obtained with those given by the fusion method. In these solutions the phosphoric acid was determined both by our method and by the suggested shaking method. The results are as follows:

Comparison of phosphoric acid determinations in soils by different methods, under varying conditions.

Soil No.	Gravimetric method precipitated at—			Volumetric method, shook 30 minutes.
	40° C.	65° C.	80° C.	
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*
883	0.057	0.055	0.055	0.052
884	.065	.065	.063	.055
885	.103	.104	.103	.098
886	.081	.082	.080	.075
887	.095	.095	.096	.088

Discussion of Results and Methods.

By a study of the results in the table it will be found that, with the exception of soil No. 2, the average results on total phosphoric acid are higher by the gravimetric than by the volumetric method. These divergences are due most probably to the small amounts of iron, alumina, or silica, or all three, carried down with the white precipitates when magnesia mixture is added, as pointed out by the associate referee in the Journal of the American Chemical Society[a] and by others elsewhere. For this reason, and for the further reason that the volumetric method precipitated all phosphoric acid in the four official samples, and in about three hundred other determinations of soil samples made by the associate referee, it is thought that the

[a] Vol. 25, p. 493.

volumetric method is preferable to the gravimetric in the determination of total phosphoric acid in soils. Also the volumetric is much shorter than the gravimetric method.

RECOMMENDATIONS.

It is recommended:

(1) That the volumetric method for the determination of total phosphoric acid in soils be made an optional method.

(2) That a study of different solvents for total phosphoric acid in soils be taken up for investigation next year.

The PRESIDENT. Are there any remarks to be made on phosphoric acid?

Mr. ROBISON. We have had trouble in Michigan with a certain class of phosphates which when treated with a sufficient amount of citrate of ammonia go completely into solution. Some experiments show that from a half of 1 per cent to 1 per cent is insoluble by the official method when 2 grams are used, whereas if 1 gram is used there will remain practically no phosphate undissolved. With a precipitated phosphate that is put out by the Michigan Carbon Works of Detroit, if we use 2 grams and 100 cc of citrate of ammonia of 1.09 specific gravity varying percentages are undissolved. The manufacturers regard this as quite an important point, as they think the present method misrepresents their goods.

The PRESIDENT. This brings up an old question with which we have been having trouble for some years and I do not know of any disposition that can be made of the matter unless this single substance be singled out and treated differently from any other phosphate. The question might be referred to the referee for investigation as to what action shall be taken.

Mr. HUSTON. For the consolation of our friend from Michigan I would say that I have a beautiful curve on this subject which I have had for about ten years. The material has not been published except in a fragmentary way, but I have quite complete data in the laboratory and shall be glad to give any information that I can on the subject. So far as making this a separate subject is concerned I would say that it really occupies a peculiar position as precipitated phosphate is probably as near reverted phosphoric acid as anything found in the trade. The method of preparation is exactly that of theoretically reverted phosphoric acid, I understand, so it practically is reverted.

Mr. WILEY. I wish to submit the following letter received from Mr. Hilgard of the California station under date of October 28, 1903:

At a late meeting of the Association of Official Agricultural Chemists the subject of an official method for the determination of available phosphoric acid in Thomas slag was dropped, on the ground that not a sufficient amount of the slag was being used in the United States; but now the use of the slag in California is extending rapidly, amounting last year to over 1,500 tons, and as our fertilizer law requires us to use the official method, we are in a predicament. As I understand it, the citrate method has

by default remained the official one, while in Europe it is almost universally discarded for the citric acid method, which corresponds more nearly to actual experience. Now, the slag comes with a certificate of available phosphoric acid according to the European method, while we are supposed to use here the one that has been discarded, and thus put the importer in jeopardy of the law. I would suggest, therefore, that this matter be taken up again at the coming meeting and referred to the proper committee for consideration.

The reason for recommending the use of Thomas slag on our soils is simply this: The arid soils are so universally rich in lime carbonate that reversion of superphosphate occurs within the first 3 or 4 inches of the surface soil, hence to bring the phosphate within reach of the roots of our deep-feeding plants it must be deeply plowed in to be effective. But the same plowing will also make the slag phosphate effective, and that being the cheaper source I recommend its use on the basis of European experience. * * * Moreover, it is especially adapted to the limonite soils of the Hawaiian Islands, in which the soluble superphosphate rapidly reverts to the same inert condition in which it is already contained, and very abundantly, in the basaltic soils of Oahu and Hawaii. Quite similar soils also occur in California and Oregon, so the question of the Thomas slag is of considerable interest to the Pacific coast and should be taken up and settled by the association.

I wrote to Mr. Hilgard, citing the proceedings of this association where the matter had been discussed, and quoted the suggestion made by a gentleman who has had a great deal of experience in this matter, to the effect that the establishment of fineness for the basic slags together with an estimate of the total phosphoric acid might be satisfactory to all concerned. Professor Hilgard telegraphed as follows:

Our station fully agrees to the standard of fineness and total phosphoric acid for Thomas slag.

I think it might be well for the referee to be asked to again consider the advisability of establishing some special method of valuing these slags. I know the danger of permitting a valuation of this kind, but it is just as fair to the slag as it is to the bone, because we do determine the total phosphoric acid in bone. Whether the State allows it in trade values or not is another question, but the official method does determine phosphoric acid in bone. We also determine the total phosphoric acid in all phosphoric fertilizers, and there is no reason why that plan should not be adopted for basic slag. Then if the degree of fineness be considered in conjunction with the total phosphoric acid as a factor in estimating its availability it appears that a conclusion satisfactory to all concerned will be reached.

Since this meeting began Mr. Huston has again called my attention to the fact, which he observed, and was one of the first to observe in his earlier service under my direction at Purdue, namely, that fine-ground bone goes into progressive solution in the ammonia citrate solution with which reverted phosphoric acid is estimated, and the amount which goes into solution is a factor or function determined by fineness, the strength of the solution, the temperature, and the time of digestion. So that any amount, almost, of the finely ground bone

could be rendered available by this treatment. Now, the same thing is true of basic slag. By adopting the European citric-acid method as the standard for valuation you can practically, by continuing the application or varying the strength or temperature, bring the whole of the basic slag into solution and render it thus apparently available. Or, by varying the conditions, you may reverse the reaction to a certain extent.

It is also well known that basic slag is far more available for plant food, as a rule, than an equal amount of phosphoric acid in the form of tricalcium phosphate undissolved, although both may be equally insoluble in water, the basic slag having a different chemical composition and therefore being more available for the nourishment of a plant. In connection with one of my assistants, I presented to this association several years ago some papers on the determination of the quantity of phosphoric slag really present in the form of tetra-calcium phosphate and the system of its crystallization. When we consider the composition of a basic slag and remember that it is a mixture of free lime, iron to some extent, tetra-calcium phosphate, with perhaps a trace of tricalcium phosphate, and many other substances, it is seen that we have a very complicated material with which to deal. Nevertheless, though the consideration of it was formerly dropped by the association because it was not coming into the country to any extent and was not largely used as a fertilizer, in the light of the statement made by Mr. Hilgard we must now reverse that opinion and recognize that basic slag is again becoming commercially important in this country.

I therefore move that the referee on phosphoric acid be instructed to reconsider the question of the valuation of basic phosphatic slags, especially the recommendation to establish a standard based on the total phosphoric acid and degree of fineness.

The PRESIDENT. Perhaps Mr. Wiley would include the precipitated and the Redonda phosphates.

Mr. WILEY. I have no objection to that.

Mr. HUSTON. There is one point in connection with these slags, which seems to have had the special attention of our friends in Europe who determine availability by citric acid alone, that we ought to consider. While the tricalcium phosphate goes into solution progressively under different conditions of temperature with basic slag, the amount of phosphoric acid going into solution increases for a time with citric acid. It then decreases, and if the action of 1 or 2 per cent citric acid is continued long enough a point is reached where there will be a very large amount of lime, iron, manganese, and the other constituents of the slag in solution, and no phosphoric acid at all is left, it being entirely precipitated.

I do not believe that any method which under varying conditions of

temperature will give a maximum at different times and finally give nothing is a suitable method for any commercial material, and it seems to me that the matter should be very seriously considered. The fact that phosphoric acid will not remain in solution in citric acid if the action be sufficiently prolonged has not received much attention. Moreover, in dealing with citrate of ammonia it must be remembered that the solution must be much more dilute, or, rather, with two grams of slag and 100 cc of liquid the amount dissolved is increased very rapidly by simply adding water. We do not make any progress of real value unless these two simple facts are considered.

Furthermore, there are other slags which you will be called upon to value later. They are not the Bessemer crucible slags, but the open-hearth slags, which are now made in very large quantities near St. Louis, and as the Bessemer ores are exhausted the phosphoric acid in these slags will rise until they finally become an article of commerce. They differ somewhat from the others in that they contain a large amount of silica, which may make trouble. Enormous quantities of these open-hearth slags will be produced before long, and some of them already contain 10 per cent of phosphoric acid, which almost places them in the commercial class.

The PRESIDENT. The resolution offered by Mr. Wiley is before the convention.

The resolution was adopted.

Mr. PENNY. I wish to offer the following resolution on soil, if it is in order:

Resolved, That the referee on soils be requested to make a thorough examination of the methods described in Bulletin 22 of the Bureau of Soils for the determination of available plant food.

Adopted.

Mr. CAMERON. Mr. President, I wish to say that a misunderstanding has evidently gone forth, and is in the minds of the majority of the gentlemen here, in regard to the purpose of Bulletin No. 22. It was not maintained in that bulletin that the procedure there described would determine the available plant food in the soil in the sense in which "available plant food" is supposed to be determined by the conventional procedures. The analytical methods used were given as an explanation of our work and an earnest of good faith. They were developed for our own particular purposes, although of course we can not have the slightest objection to anybody using them.

Our object in the beginning of the work was to develop methods which would enable us to determine exceedingly small amounts in solution and then with these methods to develop a procedure which would enable us to follow what changes might be taking place in the soil from day to day. It was found impracticable in the field work described in Bulletin No. 22 to make the examinations daily, but we

did as well as we could by making them every week or ten days during the growing season.

I wish to state distinctly to this convention, so that there may be no misunderstanding, that while there is no objection to anyone trying these methods, the authors of Bulletin No. 22 do not and never have proposed a procedure for the determination of the fertility of soils. Bulletin No. 22 shows that the procedure we followed would not work; it does not determine the fertility of the soil, and in this regard is absolutely without analogy to other proposed methods, such as the methods to which Mr. Wiley has referred, those Mr. Hilgard has suggested, or the "official" method. Our methods were intended for an entirely different purpose, and the Bureau of Soils has never supposed they would be considered as proposed for the same purpose as the official method.

Now we may some time propose a method for determining fertility—Professor Whitney and I have been investigating one—but it is something entirely different in character from the work described in Bulletin No. 22. I will say once more that the procedures described in Bulletin No. 22 are not, and there is nothing in the bulletin which can be tortured into saying they are, methods for the determination of the fertility of the soil in the sense in which conventional methods are so used.

The PRESIDENT. We will now take up the report on sugar, and in justice to the referee, Mr. Tolman, I wish to say that he took up the work late in the year, at my request, upon the resignation of Mr. Spencer.

REPORT ON SUGAR.

By L. M. TOLMAN, *Referee.*

The work on sugar this year has been very limited. Mr. Munson has continued the work on reducing sugar commenced last year, and will make a report at this meeting. Mr. Davoll, associate referee on beet-sugar methods, through some misunderstanding, did not send in any report to the meeting, but has since made a few suggestions in a letter to the referee as to work for next year, which are as follows:

I have been considering the matter of taking up several lines of investigation after the campaign is over, continuing the work on raffinose and starting others. We lay great stress upon raffinose determinations in our factory, as the carbohydrate is present to a considerable extent in Michigan beets. As the campaign progresses I shall be able to suggest some cooperation work after watching our own laboratory work during that time.

Regarding additional work, I would suggest investigations upon the determinations of sucrose and raffinose in the presence of reducing sugars. J. Wortman[a] has worked out a formula, but I believe that it is not generally accepted as correct.

Another thing that we need is a practical method for the direct analysis of the beet by the hot-water-digestion method in the tare room, capable of handling up to 500 samples per day, with the minimum of labor. I am inclined to favor the Sachs-Le Docte method, but look for a simplified manipulation as regards the digestion. An instantaneous cold method would do if a uniform and continuous supply of fine pulp could be had.

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[a] Spencer's Handbook, p. 110; also Zeit. Rübenzucker-Ind., **39**: 766.

Mr. Munson's paper being in the nature of a report of progress, he makes no recommendations except that a continuation of the work is very desirable. It is hoped that by next year some definite results may be offered the association, which are especially needed, as at this time we have two distinct methods of procedure for the Allihn method which give different results, and yet the same tables are used.

RECOMMENDATIONS.

(1) The question of the analysis of such products as molasses has been under discussion lately, as the methods now in use require certain modifications before they can be applied, and the question has been raised as to whether it would not be well to appoint another associate referee on sugar to take up the subject of the application and modification of our present methods to apply to such products. I think that the only way in which we can accomplish much in sugar work, as in most of the other lines of work in this association, is through individual effort, and would recommend that such an associate be appointed. I believe Mr. Sawyer, who is much interested in this subject at the present time, will give an outline of the work he has been doing on molasses methods and make some suggestions as to work for next year.

(2) The international association for the unification of sugar methods has at the present time under discussion some changes in the copper solution used to determine reducing sugars, and I would recommend that the referee be instructed to communicate with the committee which has this work in charge with the idea of cooperating with them on this subject.

(3) The recommendations involving changes in official and provisional methods on sugar and several minor changes in Bulletin No. 46 which were made at the last meeting and printed in the proceedings of that year (1902) were overlooked by the committee on recommendations of referees, and should be acted upon at this meeting. (See report of Committee B, p. 230.)

The PRESIDENT. Before proceeding further with the discussion of the sugar report, I wish to introduce Doctor Needham, of the Columbian University.

ADDRESS BY PRESIDENT NEEDHAM, COLUMBIAN UNIVERSITY.

Mr. PRESIDENT AND GENTLEMEN: It gives me great pleasure on behalf of the University to extend a cordial greeting to this association of distinguished workers in one of the most important fields of science. We desire to put at your service every facility we have that will add to your comfort and the success of your meeting. These recurring gatherings add to our obligations; for we feel that your work is a large contribution to every institution of learning, and because of our fortunate location we receive a direct and special benefit from your discussions. I should esteem it a great pleasure to listen to all of the able papers and remarks offered at this meeting.

In view of your annual gatherings here, it may not be out of place for me to say that the University is looking toward a larger life, better buildings, and equipment. We have secured a site on Seventeenth street, south of the State Department, and fronting on the President's park. This is in the immediate vicinity of the Agricultural Department, with its fine equipment of men and laboratories, which is working out great problems of public interest, and may also become helpful to many graduate students and men engaged in original research. In this location we hope to erect a well-equipped chemical laboratory and conduct graduate work of a high order. In this advanced work we shall desire to avail ourselves of the assistance of many scientists who are developing knowledge along these lines. We also hope that these

buildings and laboratories, when erected, may furnish a temporary home and workshop for investigators who come to Washington for a longer or shorter period to avail themselves of the special advantages for research work existing in this city. For general assemblies like the present, we shall have better accommodations to offer, and they will be quite as convenient in all respects as the present location. It will take a little time to work out these plans and complete these buildings, and in the meantime the present building and equipment are yours while you are in the city and can use them.

There is one thought in my mind in reference to your work that I wish I might clearly express, and that is, the intellectual and spiritual benefit which is coming to mankind as a result of your labors. In the wonderful discoveries of science we are apt to think only of the immediate discovery and overlook the greater results that flow from it. You chemists are at the heart of things. You are discovering facts and laws which, in their natural operation, have far-reaching effects upon human life. The theory of evolution has fought its way and has been accepted. All recognize to-day that the things that are have come out from the past, formed and adapted by the environment in which the life found itself. The natural law of adaptation has worked and is working out the sublime objects of its author and human life has risen to higher and higher planes and to greater usefulness. But in these later years another thought has come to the world's students. If the environment has such an effect upon life, then why not change the environment and make it better, accepting the natural law the scientists are working to improve the conditions of life, and in this way are producing and will in the future produce a higher and a better life for man. In mechanics, after the machine, comes the search after more favorable conditions—the reduction of friction in order to secure better and greater results. So, having discovered a great law of life, the aim of the scientists is to improve the conditions, to relieve all unnecessary strain upon vital forces in order that the intellectual and spiritual life of man may have greater freedom and power of action. The man who finds himself upon a poor and unproductive soil need not take a long journey to new fields, but through the discoveries of chemistry he may make his land productive. Not only that, but he may know the products best adapted to the soil and climate in which he lives. Does the manufacturer find his profits diminishing and his capital threatened by the expense or the difficulties of production, the chemist will tell him how to increase the quality and decrease the expense. In almost every line of human activity where physical facts and forces are employed the chemist has become an indispensable man; and thus he is adding to the wealth of the nation and bringing within the reach of many, at a reasonable cost, the things which supply the ever increasing wants of our higher civilization.

Not only is the environment being changed for the better but by chemical investigations and experiments the foods we eat and the drinks we drink are purified, and their adaptation to our varying wants is made known. Thus the body is protected against harmful influences and relieved from much of the strain on the vital forces caused by the effort to overcome the bad effects of injurious matter; and by this selection, following the natural law, the best growth and development of the physical nature is secured. Thus is the work of the chemist making the conditions of physical life more favorable, and thereby he is making it possible to develop to a higher degree the intellectual and spiritual life. This result must always be regarded as the final goal of human life, and everything that tends to such development and enlargement is a noble work.

In the early history of this country the continent was a wilderness, but by the intelligent energy of our people, our frontiers have been driven back upon the undiscovered country and the wilderness has given up to civilized man its vast resources. So in the world of knowledge the great body of natural laws and facts was unknown until the scientists, by persistent effort and struggle, had driven back

the boundary line of ignorance, and large fields in the realm of truth had been uncovered.

In the discovery of natural forces and laws and the rearrangement of matter to produce new and better conditions, man is working for the rejuvenation of man. Not by dreaming, nor by superstition, nor by the works of superstition, but by the direct, persistent, sincere, and fearless work of the student in the realms of nature is discovered that light which "shineth in darkness;" and then is brought into the great treasure house of humanity the facts and knowledge which are indeed "both new and old."

I congratulate you upon the splendid work you are doing, and trust that there will be many returns of the meeting of this association in the halls of this university.

The PRESIDENT. I am sure I express the thought of the association when I extend to President Needham our thanks for the words he has spoken. We appreciate what he has said, especially coming as it does from a man of his attainments and position.

We now come to the recommendations made by the referee on sugar. As he proposed, the recommendations made by the referee of last year will be referred to the committee having sugar under consideration for final adoption or other action. Are there any other papers on sugar?

Mr. SAWYER. I wish to submit a brief paper on methods of molasses analysis.

METHODS FOR THE ANALYSIS OF MOLASSES.

By H. E. SAWYER.

In examining several hundred samples of molasses during the last two years, I have found it impossible to obtain sufficiently accurate results by use of the analytical methods given in Bulletins Nos. 46 and 65. For the analysis of goods of fairly high quality, containing little coloring matter or other organic non-sugar, these methods are quite satisfactory; and for goods of lower grade they still are satisfactory, provided that approximate results are sufficient. But for the accurate analyses, such as ought to precede the acceptance of large lots of the low-grade molasses commonly worked up into alcohol, they are entirely inadequate.

The commercial chemists of our cities, on whose tests this molasses is usually bought and sold, have proceeded, in consequence of the lack of a proper official method, to follow each his own will and way in making analyses of these goods, with the result that satisfactory agreement between two analysts is very rare. In case of disagreement, who is to decide which chemist is in the right? And in case of serious disagreement and reference to the courts, how can an equitable decision be given in the absence of a reliable and official method? In consequence of this state of affairs, it is desirable for large buyers of molasses that the Association of Official Agricultural Chemists come to their assistance, as it did to the aid of the tanners a few years since, in developing and recommending analytical methods of suitable accuracy.

As I already have indicated, the inadequacy of the present methods in testing low-grade molasses is due to the intense coloration of such goods. To clarify a very dark and thick molasses sufficiently for polarization without the use of bone black may require 50 or 60 cc of basic lead acetate if the Ventzke normal weight is dissolved and made up to a volume of 100 cc, as recommended on page 39 of Bulletin No. 46. The bulk of the lead precipitate so produced concentrates the sugar solution and raises the polarization unduly. Moreover, the excess of lead remaining in the solution acts on the specific rotary power of the sugars and raises

the polarization still further. I have known extreme cases where the direct polarization obtained with solutions so prepared was about a third in excess of what I believed to be the true value. That is to say, a reading of 22° was obtained when a value of 17° or 18° would have been nearer correct. In addition to its effect upon the direct polarization, the presence of a large excess of lead interferes with the inversion of a sugar solution and with the correct determination of the invert polarization and the sucrose.

To avoid difficulties of this sort, experiments were made during the last two years in the direction of using more dilute solutions than are recommended in the association's present methods. I dissolved the normal weight and made up to 500 cc; with this lower concentration I succeeded in getting filtrates light enough for polarization, using no more than 10–15 cc of basic acetate. The decrease in the bulk of the precipitate and in its influence upon the concentration of the solution, and the absence of the troublesome excess of lead from the solution, gave results nearer to the true polarization values and facilitated inversion.

Of course the polariscope readings on the dilute solutions in tubes of regulation length must be multiplied by five in order to obtain the actual polarization values, and it may be objected that this involves the multiplication of observation error. In fact, however, when the sum of five consecutive readings is taken as indicating the actual polarization, the probable error is very slight. With the triple field saccharimeter, showing clearly a difference of 0.05°, it may be a not uncommon experience to have five such readings agree absolutely; and in extreme cases of variation the probable error is surely smaller than the certain error involved in working on the lines of the present method.

To illustrate the closeness with which a polarization may be determined in the dilute solution I give the readings obtained in one of many tests upon this point.

	Degrees.
I. Sucrose solution containing approximately 6.5 grams per 100 cc. Polarized in 200 mm tube (five readings taken)	25.00
	24.95
	25.05
	25.00
	25.05
Average	25.01

	Degrees.	Degrees.
II. Sucrose solution exactly one-fifth the concentration of that used in No. I. Polarized in 200 mm tube (two sets of five readings each)	5.00	5.00
	4.95	4.95
	5.00	5.00
	4.95	5.05
	5.05	5.00
Sums	24.95	25.00
Mean errors of sums	±.09	±.075

It must be recognized by everyone who has had experience in molasses analysis that these errors are much smaller than those which attend the attempt to polarize the dark solutions prepared at the concentration of 26 grams to 100 cc.

It may be asked whether the polariscopic readings may not be materially affected by the change in the specific rotary powers of the sugars of molasses, due to the lowering of the concentration from 26 grams to 100 cc to 26 grams to 500 cc. I have found in practice that no effect of this sort is noticeable, and have calculated and here present the theoretical effect of such a change of concentration for a mixture of pure sucrose and invert sugar.

It may be assumed for the purpose of this calculation that the sirup contains 26.923 per cent of sucrose and an equal amount of invert sugar. These proportions are practically the same as those of low-grade molasses, and they give, according as the normal weight of 26 grams is dissolved to 100 or to 500 cc, concentrations of 7 or

1.40 grams per 100 cc for both sugars. The specific rotary powers and the corresponding Ventzke polarizations for the two concentrations are as follows:[a]

Polarizations with varying concentrations.

Concentration.		Sucrose.		Invert sugar.		Resultant direct polarization.
Sirup.	Sugar.	Specific rotation.	Polarization.	Specific rotation.	Polarization.	
		Angular degrees.	*Ventzke degrees.*	*Angular degrees.*	*Ventzke degrees.*	*Ventzke degrees.*
26:100	7.00:100	66.484	26.917	−19.910	−8.060	18.857
26:500	1.40:100	66.446	26.901	−19.708	−7.979	18.922
Difference.		.....	.016	.....	— .081	.065

Here, therefore, as in the observation, the possible error is materially smaller than the certain inaccuracies which attend the present method of molasses analysis.

Of course I do not claim that it is possible by using the dilute solution to determine with absolute accuracy the sucrose of a dark molasses. It is believed, however, that it is possible to insure increased accuracy, and that the method as outlined is a safe one, though still capable of further development. With this belief, and in the interest of the large molasses users I ask for it the consideration of the association.

Mr. Munson. I have no report to make, but as associate referee on sugar wish to say a few words on the work done during the past year. As stated at the last meeting, this work has for its purpose the obtaining of a more satisfactory alkaline tartrate solution, the idea being to get a solution that could be applied to all sugars. Considerable work has been done along this line during the past year, but at the present time there is nothing definite to report except that I have succeeded in getting a solution that has very much less effect on cane sugar than any of the solutions in use, even less than the Soxhlet solution, which is the most dilute and has the least effect on cane sugar of any solution now recognized. This solution requires 50 grams of caustic soda and 173 grams of rochelle salts per 500 cc. I have reduced the caustic soda to 24 grams per 500 cc and increased the rochelle salts to 275 grams. When working with a 20 per cent solution of pure cane sugar and using the Soxhlet method the reduction amounts to 50 mg of cuprous oxid, while with the new solution the reduction amounts to something less than 17 mg, about one-third of the effect so far as the action on cane sugar is concerned. With a 10 per cent solution of cane sugar, using the Soxhlet method, the reduction amounts to about 40 mg, while with the new solution the reduction is less than 11 mg. The idea has been to get a weak solution, one that will not reduce spontaneously and yet will have the least effect on cane sugar. It has been somewhat difficult to work this out. I think it would be a good

[a] Optical Rotation of Organic Substances. Landolt-Long. 1902. Sucrose, p. 465; invert sugar, p. 592.

idea to continue this work another year, and if we get a satisfactory solution for all the sugars, then to work out factors and a method for the various reducing sugars, using a given method and a given solution.

As the referee reported, there has been a committee appointed by the International Congress of Sugar Chemists, for the purpose of looking into this matter of a satisfactory solution for the reducing-sugar method, and I think it would be well for this association to authorize some cooperation.

The PRESIDENT. Is there any discussion.

Mr. WILEY. Some of our members may not know what the principal things are that the international committee has done. In the first place, it has recommended that the temperature at which all apparatus is standardized should be changed from 17.5° C to 20° C, as it is recognized that the former is too low. I was in favor of 22.5°, which is still 2° below the average temperature of our laboratories during the summer. We have made some interesting observations on this point, having kept the temperatures of polarization at the ports of New York and Boston every day for two years and have found the average to be over 23° C. It is seen that the adopted temperature is still far below our standard, but it is much better than before.

The next most important resolution of the international committee is that all polarizations shall be made at the temperature at which the instruments are graduated, but each country is allowed to have its instruments graduated at its own standard temperature if it be higher or lower than 20°. Thus the errors due to graduating instruments at one temperature and making polarizations at another are eliminated. I think that is the most important work of the international committee.

The third is that a true cubic centimeter flask shall be used with 26 grams of sugar instead of 26.048 grams with the Mohr flask. That eliminates an element of discord which never should have entered into sugar analysis at all.

These are the most important resolutions of the committee. It is working all the time and is now preparing one standard solution of sugar. Thus this principle, which started with the organization of this association, has permeated all the world, and instead of using instruments standardized by everyone who uses them, there is one source from which standard sugars can be obtained, that is, from the president of the international committee. Each country is allowed to make its own standard sugar if it wants to, but it is far better to get it from one source. We have received a package for the United States, a part of which has been given to the National Bureau of Standards. The members of this association ought to know that the National Bureau of Standards will standardize instruments free of cost for all Government institutions, and I think that the agricultural

experiment stations come under that category. It is most important in sugar analysis to know that the polariscope is right. While formerly the differences in polarization often averaged as much as 0.8 per cent the comparative data to-day rarely differ more than 0.1 per cent, which shows what has been accomplished by the adoption of these uniform methods.

The PRESIDENT. I understand that the committee on food standards is ready to report.

REPORT OF THE COMMITTEE ON FOOD STANDARDS.

THE PRESIDENT AND MEMBERS OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS: On behalf of the standing committee on food standards, I desire to report that Congress, by an act approved March 3, 1903, made further provision "to enable the Secretary of Agriculture in collaboration with the Association of Official Agricultural Chemists, and such other experts as he may deem necessary, to establish standards of purity for food products and to determine what are regarded as adulterations therein, for the guidance of the officials of the various States and the courts of justice."

Acting under commission from the Secretary of Agriculture and the authority conferred by this association, the committee has, during the past year, prepared a further revision of several of the schedules to which your attention and that of trade and commercial interests concerned have already been called, and has conducted a voluminous correspondence respecting the definitions and standards proposed.

Three meetings have been held, of an aggregate duration of sixteen days, during which numerous hearings have been given to trade interests requesting an opportunity for conference and for discussion of the definitions and standards. It is a pleasure to report that, while naturally solicitous lest hasty and ill-considered action might needlessly disturb the vast business interests concerned, the representatives of those engaged in food manufacture and commerce have expressed a cordial approval of the movement as conducive to the welfare of both producer and consumer.

Your committee recommends to the Secretary of Agriculture for approval at this time standards for certain articles belonging to the meat, milk, sugar, condiment, and cocoa schedules. They are hereto appended:

Before the adoption of any schedule, it has been submitted to the manufacturing firms and the trade immediately interested for criticism and, when requested by them, conferences for discussion have been arranged. Certain questions have arisen in the discussion of these standards relative to several substances sometimes used as preservatives or coloring matters; in the judgment of the committee these questions can most satisfactorily be treated in connection with Schedule III, Preservatives and coloring matters, and recommendations have therefore been deferred pending the consideration of that schedule.

For the primary definitions and standards and for the compilations of data for standards and constant assistance in the revision of the schedules, the committee is greatly indebted to the following persons:

Charles D. Woods, Ph.D., director of the Maine Agricultural Experiment Station, Orono, Me., referee on meat and its products; L. L. Van Slyke, Ph.D., chemist of the New York Agricultural Experiment Station, Geneva, N. Y., referee on milk and its products; Charles A. Crampton, M. D., chemist of the Bureau of Internal Revenue, referee on beverages, including cocoa and cocoa products; A. L. Winton, Ph.B., chemist of the Connecticut Agricultural Experiment Station, New Haven, Conn., referee on condiments.

The committee is also indebted to others for information and helpful suggestions, which will be more specifically acknowledged in a report of its work to be later submitted.

Very respectfully,

WILLIAM FREAR.
E. H. JENKINS.
M. A. SCOVELL.
H. A. WEBER.
H. W. WILEY.

STANDARDS OF PURITY FOR FOOD PRODUCTS

Whereas the Congress of the United States by an act approved June 3, 1902, authorized the Secretary of Agriculture to establish standards of purity for food products; and

Whereas he was empowered by this act to consult with the committee on food standards of the Association of Official Agricultural Chemists and other experts in determining these standards; and

Whereas he has in accordance with the provisions of the act availed himself of the counsel and advice of these experts and of the trade interests touching the products for which standards have been determined, and has reached certain conclusions based on the general principles of examination and conduct hereinafter mentioned;

Therefore, I, James Wilson, Secretary of Agriculture, do hereby proclaim and establish the following standards for purity of food products together with their precedent definitions as the official standards of these food products for the United States of America.

JAMES WILSON.

WASHINGTON, D. C., *November 21, 1903.*

PRINCIPLES ON WHICH THE DEFINITIONS AND STANDARDS ARE BASED.

The general considerations which have guided the committee in preparing the definitions and standards for food products are the following:

1. The main classes of food articles are defined before the subordinate classes are considered.
2. The names of the various substances for which standards are proposed are defined.
3. The definitions are so framed as to exclude from the articles defined substances not included in the definitions.
4. The definitions include, where possible, those qualities which make the articles described wholesome for human food.
5. A term defined in any of the several schedules has the same meaning wherever else it is used in this report.
6. The names of food products herein defined usually agree with existing American trade or manufacturing usage, but where such usage is not clearly established or where trade names confuse two or more articles for which specific designations are desirable, preference is given to one of the several trade names applied.
7. Standards are based upon data representing materials produced under American conditions and manufactured by American processes or representing such varieties of foreign articles as are chiefly imported for American use.
8. The standards fixed are such that a departure of the articles to which they apply, above the maximum or below the minimum limit prescribed is evidence that such articles are of inferior or abnormal quality.
9. The limits fixed as standard are not necessarily the extremes authentically recorded for the article in question, because such extremes are commonly due to abnormal conditions of production and are usually accompanied by marks of inferiority or abnormality readily perceived by the producer or manufacturer.

FOOD DEFINITIONS AND STANDARDS.

I. Animal Products.

A. MEATS AND THE PRINCIPAL MEAT PRODUCTS.

(a) Meats.

Definitions.

1. *Meat* is any sound, dressed, and properly prepared edible part of animals in good health at the time of slaughter. The term "animals," as herein used, includes not only mammals, but fish, fowl, crustaceans, mollusks, and all other animals used as food.
2. *Fresh meat* is meat from animals recently slaughtered or preserved only by refrigeration.
3. *Salted*, *pickled*, and *smoked meats* are unmixed meats preserved by salt, sugar, vinegar, spices, or smoke, singly or in combination, whether in bulk or in packages.

Standard.

Standard meat, fresh meat, and salted, pickled, and smoked meats are such as conform respectively to the foregoing definitions.

(b) Manufactured Meats.

Definition.

1. *Manufactured meats* are meats not included in definitions 2 and 3, whether simple or mixed, whole or comminuted, in bulk or packages, with or without the addition of salt, sugar, vinegar, spices, smoke, oils, or rendered fat.

Standard.

Standard manufactured meats conform to the foregoing definition. If they bear names descriptive of composition, they correspond thereto, and when bearing such descriptive names, if force or flavoring meats are used, the kind and quantity thereof are made known.

(c) Meat Extracts, Meat Peptones, etc.

(Schedule in preparation.)

(d) Lard.

Definitions.

1. *Lard* is the rendered fresh fat from slaughtered, healthy hogs.
2. *Leaf lard* is the lard rendered at moderately high temperatures from the internal fat of the abdomen of the hog, excluding that adherent to the intestines.

Standard.

Standard lard and standard leaf lard are lard and leaf lard, respectively, free from rancidity, containing not more than one (1) per cent of substances, other than fatty acids, not fat, necessarily incorporated therewith in the process of rendering, and standard leaf lard has an iodine number not greater than sixty (60).

Definition.

3. *Neutral lard* is lard rendered at low temperatures.

B. MILK AND ITS PRODUCTS.

(a) Milks.

Definition.

1. *Milk* (*whole milk*) is the lacteal secretion obtained by the complete milking of one or more healthy cows, properly fed and kept, excluding that obtained within fifteen days before and five days after calving.

Standard.

Standard milk is milk containing not less than twelve (12) per cent of total solids and not less than eight and one-half (8.5) per cent of solids not fat, nor less than three and one-fourth (3.25) per cent of milk fat.

Definitions.

2. *Blended milk* is milk modified in its composition so as to have a definite and stated percentage of one or more of its constituents.
3. *Skim milk* is milk from which a part or all of the cream has been removed.

Standard.

Standard skim milk is skim milk containing not less than nine and one-fourth (9.25) per cent of milk solids.

Definitions.

4. *Buttermilk* is the product that remains when butter is removed from milk or cream in the process of churning.
5. *Pasteurized milk* is standard milk that has been heated below boiling but sufficiently to kill most of the active organisms present and immediately cooled to fifty degrees (50°) Fahr. or lower to retard the development of their spores.
6. *Sterilized milk* is standard milk that has been heated at the temperature of boiling water or higher for a length of time sufficient to kill all organisms present.
7. *Condensed milk* is milk from which a considerable portion of water has been evaporated.
8. *Sweetened condensed milk* is milk from which a considerable portion of water has been evaporated and to which sugar (sucrose) has been added.

Standard.

Standard condensed milk and *standard sweetened condensed milk* are condensed milk and sweetened condensed milk, respectively, containing not less than twenty-eight (28) per cent of milk solids, of which not less than one-fourth is milk fat.

Definition.

9. *Condensed skim milk* is skim milk from which a considerable portion of water has been evaporated.

(*b*) Milk Fat or Butter Fat

Definition.

1. *Milk fat* or *butter fat* is the fat of milk.

Standard.

Standard milk fat or butter fat has a Reichert-Meissl number not less than twenty-four (24) and a specific gravity not less than 0.905 (40° C. /40° C.).

(*c*) Cream.

Definition.

1. *Cream* is that portion of milk, rich in butter fat, which rises to the surface of milk on standing, or is separated from it by centrifugal force.

Standard.

Standard cream is cream containing not less than eighteen (18) per cent of milk fat.

Definition.

2. *Evaporated cream* is cream from which a considerable portion of water has been evaporated.

(*d*) Butter.

Definition.

1. *Butter* is the product obtained by gathering in any manner the fat of fresh or ripened milk or cream into a mass, which also contains a small portion of the other milk constituents, with or without salt. By acts of Congress approved August 2, 1886, and May 9, 1902, butter may also contain additional coloring matter.

Standard.

Standard butter is butter containing not less than eighty-two and five-tenths (82.5) per cent of butter fat.

Definition.

2. *Renovated or process butter* is the product obtained by melting butter and reworking, without the addition or use of chemicals or any substances except milk, cream, or salt.

Standard.

Standard renovated or process butter is renovated or process butter containing not more than sixteen (16) per cent of water and at least eighty-two and five-tenths (82.5) per cent of butter fat.

(*e*) Cheese.

Definitions.

1. *Cheese* is the solid and ripened product obtained by coagulating the casein of milk by means of rennet or acids, with or without the addition of ripening ferments and seasoning. By act of Congress, approved June 6, 1896, cheese may also contain additional coloring matter.
2. *Whole-milk or full-cream cheese* is cheese made from milk from which no portion of the fat has been removed.
3. *Skim-milk cheese* is cheese made from milk from which any portion of the fat has been removed.
4. *Cream cheese* is cheese made from milk and cream, or milk containing not less than six (6) per cent of fat.

Standard.

Standard whole-milk cheese or full-cream cheese is whole-milk or full-cream cheese containing in the water-free substance not less than fifty (50) per cent of butter fat.

(*f*) Miscellaneous Milk Products.

Definition.

1. *Ice cream* (in preparation).

Standard.

Standard ice cream (in preparation).

Definitions.

2. *Whey* is the product remaining after the removal of fat and casein from milk in the process of cheese making.
3. *Kumiss* is mare's or cow's milk, with or without the addition of sugar (sucrose), which has undergone alcoholic fermentation.

II. Vegetable Products.

A. Grain products.

(Schedule in preparation.)

B. Fruits and vegetables.

(Schedule in preparation.)

C. SUGARS AND RELATED SUBSTANCES.

(a) Sugar and Sugar Products.

Definition.

1. *Sugar* is the product chemically known as sucrose (saccharose), chiefly obtained from sugar cane, sugar beets, sorghum, maple, or palm.

Standard.

Standard sugar is white sugar containing at least ninety-nine and five-tenths (99.5) per cent of sucrose.

Definitions.

2. *Granulated, loaf, cut, milled, and powdered sugars* are different forms of standard sugars.

3. *Maple sugar* is the solid product resulting from the evaporation of maple sap.

4. *Massecuite, melada, mush sugar, and concrete* are products obtained by evaporating the purified juice of a sugar-producing plant, or a solution of sugar, to a solid or semi-solid consistence in which the sugar chiefly exists in a crystalline state.

5. *Molasses* is the product left after separating the sugar from massecuite, melada, mush sugar, or concrete.

Standard.

Standard molasses is molasses containing not more than twenty-five (25) per cent of water nor more than five (5) per cent of ash.

Definitions.

6. *Sirup* is the product obtained by purifying and evaporating the juice of a sugar-producing plant without removing any of the sugar.

7. *Sugar-cane sirup* is a sirup obtained by the evaporation of the juice of the sugar cane or by the solution of sugar-cane concrete.

8. *Sorghum sirup* is a sirup obtained by the evaporation of sorghum juice or by the solution of sorghum concrete.

9. *Maple sirup* is a sirup obtained by the evaporation of maple sap or by the solution of maple concrete.

10. *Sugar sirup* is a product obtained by dissolving sugar to the consistence of a sirup.

Standard.

Standard sirup is a sirup containing not more than thirty (30) per cent of water nor more than two and five-tenths (2.5) per cent of ash.

(b) Glucose Products.

Definition.

1. *Starch sugar or grape sugar* is the solid product obtained by hydrolyzing starch or a starch-containing substance until the greater part of the starch is converted into dextrose. Starch sugar or grape sugar appears in commerce in two forms, anhydrous and hydrous. In the former, the sugar is crystallized without water of crystallization; in the latter, it is crystallized with water of crystallization. The hydrous varieties are commonly known as 70 and 80 sugars; 70 sugar is also known as brewers' sugar, and 80 sugar as climax or acme sugar.

Standards.

(a) *Standard 70 sugar or brewers' sugar* is hydrous starch sugar containing not less than seventy (70) per cent of dextrose and not more than eight-tenths (0.8) per cent of ash.

(b) *Standard 80 sugar, climax or acme sugar*, is hydrous starch sugar containing not less than eighty (80) per cent of dextrose and not more than one and one-half (1.5) per cent of ash.

(c) *Standard anhydrous grape sugar* is anhydrous grape sugar containing not less than ninety-five (95) per cent of dextrose without water of crystallization and not more than eight-tenths (0.8) per cent of ash.

The ash of these standard products consists almost entirely of chlorids and sulphates of lime and soda.

Definition.

2. *Glucose, mixing glucose, or confectioners' glucose* is a thick sirupy substance obtained by incompletely hydrolizing starch or a starch-containing substance, decolorizing and evaporating the product. It is found in various degrees of concentration, ranging from forty-one (41) to forty-five (45) degrees Baumé.

Standard.

Standard glucose, mixing glucose, or confectioners' glucose is colorless glucose, varying in density between forty-one (41) and forty-five (45) degrees Baumé, at a temperature of one hundred (100) degrees F. (37.7° C.). It conforms in density, within these limits, to the degree Baumé it is claimed to show, and for a density of forty-one (41) degrees Baumé contains not more than twenty-one (21) per cent of water and for a density of forty-five (45) degrees not more than fourteen (14) per cent. It contains on a basis of forty-one (41) degrees Baumé not more than one (1) per cent of ash, consisting chiefly of chlorids and sulphates of lime and soda.

Definition.

3. *Glucose sirup or corn sirup* is glucose unmixed or mixed with sirup or molasses.

Standard.

Standard glucose sirup or *corn sirup* is glucose sirup or corn sirup containing not more than twenty-five (25) per cent of water nor more than three (3) per cent of ash.

(*c*) CANDY.

Definition.

1. *Candy* is a product prepared from a saccharine substance or substances, with or without the addition of harmless coloring, flavoring, or filling materials.

Standard.

Standard candy is candy containing no terra alba, barytes, talc, chrome yellow, or other mineral substances or poisonous colors or flavors or other ingredients injurious to health.

(*d*) HONEY.

(Schedule in preparation.)

D. CONDIMENTS (EXCEPT VINEGAR).

(*a*) SPICES.

General Definition.

1. *Spices* are aromatic vegetable substances used for the seasoning of food.

General Standard.

Standard spices are sound spices, true to name, from which no portion of any volatile oil or other flavoring principle has been removed.

Definition.

2. *Allspice or pimento* is the dried fruit of *Pimenta officinalis* Lindl.

Standard.

Standard allspice is allspice containing not less than eight (8) per cent of quercitannic acid;[a] not more than six (6) per cent of total ash; not more than five-tenths (0.5) per cent of ash insoluble in hydrochloric acid, and not more than twenty-five (25) per cent of crude fiber.

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[a] Calculated from the total oxygen absorbed by the aqueous extract.

Definitions.

3. *Anise* is the fruit of *Pimpinella anisum* L.
4. *Bay leaf* is the dried leaves of *Laurus nobilis* L.
5. *Capers* are the flower buds of *Capparis spinosa* L.
6. *Caraway* is the fruit of *Carum carvi* L.

CAYENNE AND RED PEPPERS.

7. *Red pepper* is the red, dried, ripe fruit of any species of *Capsicum.*
8. *Cayenne pepper or cayenne* is the dried, ripe fruit of *Capsicum fastigiatum* DC., *Capsicum frutescens* L., *Capsicum baccatum* L., or some other small-fruited species of *Capsicum.*

Standard.

Standard cayenne pepper is cayenne pepper containing not less than fifteen (15) per cent of nonvolatile ether extract; not more than six and five-tenths (6.5) per cent of total ash; not more than five-tenths (0.5) per cent of ash insoluble in hydrochloric acid; not more than one and five-tenths (1.5) per cent of starch by the diastase method, and not more than twenty-eight (28) per cent of crude fiber.

Definitions.

9. *Celery seed* is the dried seed of *Apium graveolens* L.
10. *Cinnamon* is the dried bark of any species of the genus *Cinnamomum* from which the outer layers may or may not have been removed.
11. *True cinnamon* is the dried inner bark of *Cinnamomum zeylanicum* Breyne.
12. *Cassia* is the dried bark of various species of *Cinnamomum,* other than *Cinnamomum zeylanicum,* from which the outer layers may or may not have been removed.
13. *Cassia buds* are the dried immature fruit of species of *Cinnamomum.*
14. *Ground cinnamon or ground cassia* is a powder consisting of cinnamon, cassia, or cassia buds, or a mixture of these spices.

Standard.

Standard cinnamon or cassia is cinnamon or cassia containing not more than eight (8) per cent of total ash and not more than two (2) per cent of sand.

Definition.

15. *Cloves* are the dried flower buds of *Eugenia caryophyllata* Thunb. (*Caryophyllus aromaticus* L.) which contain not more than five (5) per cent of clove stems.

Standard.

Standard cloves are cloves containing not less than ten (10) per cent of volatile ether extract; not less than twelve (12) per cent of quercitannic acid;[a] not more than eight (8) per cent of total ash; not more than five-tenths (0.5) per cent of ash insoluble in hydrochloric acid, and not more than ten (10) per cent of crude fiber.

Definitions.

16. *Coriander* is the dried fruit of *Coriandrum sativum* L.
17. *Cumin seed* is the fruit of *Cuminum cyminum* L.
18. *Dill seed* is the fruit of *Peucedanum graveolens* Benth. & Hook.
19. *Fennel* is the fruit of *Foeniculum vulgare* Gaertn.
20. *Ginger* is the washed and dried, or decorticated and dried, rhizome of *Zingiber officinale* Roscoe.

Standard.

Standard ginger is ground or whole ginger containing not less than forty-two (42) per cent of starch by the diastase method nor less than forty-six (46) per cent by direct inversion,[b] not more than eight (8) per cent of crude fiber, not more than eight (8) per cent of total ash, not more than one (1) per cent of lime, and not more than three (3) per cent of ash insoluble in hydrochloric acid.

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[a] Calculated from the total oxygen absorbed by the aqueous extract.
[b] Copper-reducing matters by direct inversion calculated as starch.

Definition.

21. *Limed or bleached ginger* is whole ginger coated with carbonate of lime.

Standard.

Standard limed or bleached ginger is limed or bleached ginger containing not more than ten (10) per cent of ash, not more than four (4) per cent of carbonate of lime, and conforming in other respects to standard ginger.

Definition.

22. *Horse-radish* is the root of *Cochlearia armoracia* L.

Standard.

Standard grated or ground horse-radish may be mixed with vinegar.

Definition.

23. *Mace* is the dried arillus of *Myristica fragrans* Houttuyn.

Standard.

Standard mace is mace containing not less than twenty (20) nor more than thirty (30) per cent of nonvolatile ether extract, not more than three (3) per cent of total ash, not more than five-tenths (0.5) per cent of ash insoluble in hydrochloric acid, and not more than ten (10) per cent of crude fiber.

Definitions.

24. *Macassar or Papua mace* is the dried arillus of *Myristica argentea* Warb.
25. *Bombay mace* is the dried arillus of *Myristica malabarica* Lamarck.
26. *Marjoram* is the leaves, flowers, and branches of *Origanum majorana* L.
27. *Mustard seed* is the seed of *Sinapis alba* L. (white mustard), *Brassica nigra* Koch (black mustard), or *Brassica juncea* Coss. (black or brown mustard).
28. *Ground mustard* is a powder made from mustard seed, with or without the removal of the hulls and a portion of the fixed oil.

Standard.

Standard ground mustard is mustard containing not more than two and five-tenths (2.5) per cent of starch by the diastase method and not more than eight (8) per cent of total ash.

Definition.

29. *Nutmeg* is the dried seed of *Myristica fragrans* Houttuyn, deprived of its testa and with or without a thin coating of lime.

Standard.

Standard nutmegs, ground or unground, are nutmegs containing not less than twenty-five (25) per cent of nonvolatile ether extract; not more than five (5) per cent of total ash; not more than five-tenths (0.5) per cent of ash insoluble in hydrochloric acid, and not more than ten (10) per cent of crude fiber.

Definitions.

30. *Macassar, Papua, male, or long nutmeg* is the dried seed of *Myristica argentea* Warb., deprived of its testa.
31. *Paprica* is the dried ripe fruit of *Capsicum annuum* L., *Capsicum longum* DC., or some other large-fruited species of *Capsicum*.

PEPPER.

32. *Black pepper* is the dried immature berries of *Piper nigrum* L.

Standard.

Standard black pepper is black pepper free from added pepper shells, pepper dust, and other pepper by-products, and containing not less than six (6) per cent of nonvolatile ether extract; not less than twenty-two (22) per cent of starch by the diastase method; not less than twenty-eight (28) per cent of starch by direct inversion;[a] not more than seven (7) per cent of total ash; not more than two (2) per cent of ash insoluble in hydrochloric acid, and not more than fifteen (15) per cent of crude fiber. One hundred parts of the nonvolatile ether extract contain not less than three and one-fourth (3.25) parts of nitrogen.

Definitions.

33. *Long pepper* is the dried fruit of *Piper longum* L.
34. *White pepper* is the dried mature berries of *Piper nigrum* L., from which the outer coating, or the outer and inner coatings, have been removed.

Standard.

Standard white pepper is white pepper containing not less than six (6) per cent of nonvolatile ether extract; not less than fifty-three (53) per cent of starch by the diastase method; not less than forty (40) per cent of starch by direct inversion;[a] not more than four (4) per cent of total ash; not more than five-tenths (0.5) per cent of ash insoluble in hydrochloric acid, and not more than five (5) per cent of crude fiber. One hundred parts of the nonvolatile ether extract contain not less than four (4) parts of nitrogen.

Definitions.

35. *Saffron* is the dried stigmas of *Crocus sativus* L.
36. *Sage* is the leaves of *Salvia officinalis* L.
37. *Savory, or summer savory,* is the leaves, blossoms, and branches of *Satureia hortensis* L.
38. *Thyme* is the leaves and ends of blooming branches of *Thymus vulgaris* L.

(*b*) FRUIT EXTRACTS.

(Schedule in preparation.)

(*c*) SALAD OILS.

(Schedule in preparation.)

(*d*) SALT.

(Schedule in preparation.)

E. BEVERAGES (AND VINEGAR).

(*a*) TEA.

(Schedule in preparation.)

(*b*) COFFEE.

(Schedule in preparation.)

(*c*) COCOA AND COCOA PRODUCTS.

Definitions.

1. *Cocoa beans* are the seeds of the cacao tree, *Theobroma cacao* L.
2. *Cocoa nibs, or cracked cocoa* is the roasted, broken cocoa bean freed from its shell or husk.
3. *Chocolate, plain or bitter, chocolate liquor,* is the solid or plastic mass obtained by grinding cocoa nibs without the removal of fat or other constituents except the germ.

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[a] Copper-reducing matters by direct inversion calculated as starch.

Standard.

Standard chocolate is chocolate containing not more than three (3) per cent of ash insoluble in water, three and fifty-hundredths (3.50) per cent of crude fiber and nine (9) per cent of starch nor less than forty-five (45) per cent of cocoa fat.

Definition.

4. *Sweet chocolate and chocolate coatings* are plain chocolate mixed with sugar (sucrose), with or without the addition of cocoa butter, spices, or other flavoring materials.

Standard.

Standard sweet chocolate and standard chocolate coating are sweet chocolate and chocolate coating containing in the sugar- and fat-free residue no higher percentage of either ash, fiber, or starch than is found in the sugar- and fat-free residue of plain chocolate.

Definition.

5. *Cocoa or powdered cocoa* is cocoa nibs, with or without the germ, deprived of a portion of its fat and finely pulverized.

Standard.

Standard cocoa is cocoa containing percentages of ash, crude fiber, and starch corresponding to those in chocolate after correction for fat removed.

Definition.

6. *Sweet or sweetened cocoa* is cocoa mixed with sugar (sucrose).

Standard.

Standard sweet cocoa is sweet cocoa containing not more than sixty (60) per cent of sugar (sucrose) and in the sugar- and fat-free residue no higher percentage of either ash, crude fiber, or starch than is found in the sugar- and fat-free residue of plain chocolate.

(*d*) Fruit Juices—Fresh, Sweet, and Fermented.

(In preparation.)

(*e*) Vinegar.

(In preparation.)

(*f*) Mead, Root Beer, etc.

(*g*) Malt Liquors.

(In preparation.)

(*h*) Spirituous Liquors.

(In preparation.)

(*i*) Carbonated Waters, Etc.

III. Preservatives and Coloring Matters.

(In preparation.)

The President. What disposition will the association make of this report?

Mr. Wiley. As Congress has authorized the Secretary of Agriculture to proclaim and establish standards of purity for food products for the whole country and you have commissioned this committee to

prepare them, it would seem to be entirely sufficient to receive the report and await the action of the Secretary. I might say that the Secretary of Agriculture has followed the work of the committee in its progress and is ready to affix his signature to the proclamation, now in the process of engrossing, which will make these the official standards of the United States.

Upon motion the report of the committee was received.

Mr. Wiley. We have with us a committee of the American Chemical Society on the purity of reagents, which is considering matters of great importance to this association. I move that Mr. Hillebrand, the chairman of this committee, be invited to make a statement in regard to this work.

ADDRESS OF W. F. HILLEBRAND, AMERICAN CHEMICAL SOCIETY.

I would make one correction in Mr. Wiley's statement. President Long is ex officio chairman of this committee and I, as secretary, have been delegated to present to you the object of this committee—what we desire to accomplish and what we ask of you.

The committee was appointed nearly a year ago at the meeting of the American Chemical Society in this city, having in view the extreme importance to chemists of obtaining a better quality of chemical reagents than is now generally to be had. It is unnecessary probably to dwell upon the importance of this matter to a body constituted as this one is. The committee has found it difficult by correspondence to obtain a fair exchange of views, and therefore a meeting was called to be held in this city and it has been in session during yesterday and this forenoon. The committee consists of President Long as chairman, with Messrs. Baskerville, Dennis, Talbot, and myself as fellow-members. With this committee by invitation sat Messrs. Noyes, chief chemist, and H. N. Stokes, chemist, of the National Bureau of Standards. There ensued at the start a lengthy discussion as to the manner in which the cooperation of the National Bureau of Standards might be made effective. The proposition which eventually met with favor was to the following effect:

A set of specifications and tests for chemicals in different grades having been prepared and distributed, it is hoped that chemists will generally make acceptance of their purchases conditional upon their coming up to the specifications as to purity set forth in the contract or order. Dealers or manufacturers will, it is hoped, make use of a special label, to be attached to the original package, which shall be a guaranty that the contents come up to the requirements of the published list for any particular grade. If the purchaser finds that the quality implied by the label has not been supplied, he may request the National Bureau of Standards to confirm or refute his judgment by an analysis made upon the contents of an unopened original package forming part of his purchase. The certificate of the Bureau of Standards will then be just ground for rejecting the shipment in case the quality is inferior to that guaranteed. The furnishing of reagents of extraordinary purity must be a matter for special agreement between the purchaser and dealer or manufacturer. The latter may, if he desires, have a large importation or supply otherwise obtained, sampled and bottled by an agent of the Bureau, who shall then take a sample or samples for official tests. If the results of the tests are satisfactory the bottles shall be provided with an official seal of the Bureau, bearing the date and something to indicate the particular grade of chemical as listed in the published document already alluded to. This list of tests and specifications shall be prepared, presumably, under the direction of this committee, and shall be issued only with the approval of and, if possible, under the auspices of the National Bureau of Standards. It is expected that a fee will be charged for examinations made by the Bureau.

On the basis of Krauch's book, Prüfung chemischer Reagentien, a provisional list of those chemicals which should be considered at the start has been prepared,

it being the prevailing sentiment that the compilation of specifications and tests should not be a duplication of the above work, but should be one made independently with special regard to the needs of chemists in this country and that it should be prepared in less detail.

This is a very simple outline of our aims. We desire to improve the standard of chemicals furnished chemists in this country. We realize that this can not be brought about immediately. It is a work involving a great deal of labor and a great deal of correspondence, and one which demands the cooperation of chemists in all lines of chemical work throughout the country. The details of the scheme are, of course, yet to be worked out. The first step is to prepare a list of the specifications, tests, and chemicals that shall be included in this. It has been thought that at the start it would not be wise to reduplicate Krauch's large book with all the chemicals there listed and the details therein given; but to prepare a list which shall meet the principal needs of the chemists of this country. And therefore a provisional list of chemicals has been made out, which is subject to revision or addition, and this list has been subdivided among the members of the committee, who are requested to correspond with experts throughout the country and with dealers in this country and abroad to find out what grades of chemicals shall be specified and to what degree of purity each grade must conform. This is a work involving a great deal of labor and it will take several years to accomplish it entirely. It is a thing which must begin in a small way and gradually expand.

It is earnestly hoped that this association will appoint a committee to cooperate with the committee of the American Chemical Society, the Association of Official Agricultural Chemists' committee having as its work (1) the specification of those chemicals which are used particularly in its work; (2) the tests of these reagents in their different grades—if more than one grade is used; (3) the requirements that these reagents must meet in order to satisfy the demands; and (4) that your committee will report results of its deliberations to our committee, so that we may use your results in furthering the aims of this work. [Applause.]

The PRESIDENT. I feel sure that the association will do all it can to further this work and perhaps some action will be taken before our adjournment.

Proceeding with the order of business, the next report is that on ash. Mr. Fraps, the referee, is not present but his report will be presented by the secretary.

REPORT ON ASH.

By G. S. FRAPS, *Referee.*

The referee accepted the position with some reluctance last spring, upon the resignation of Mr. E. G. Runyan, the original appointee, since he had served in this capacity twice before. Various changes during the year have interfered with the work and it is regretted that there is not more to report at this meeting.

The work for the year was confined to a study of methods for the determination of sulphur and sulphates. About seven chemists having signified their willingness to take part in the work, samples were prepared and sent out with the following directions:

DIRECTIONS FOR WORK.

The samples consist of cowpeas and cotton-seed meal for the total sulphur determination, and sorghum for the sulphate work. Mix each sample thoroughly before analysis. Make at least two determinations by each method. In reporting give weights of precipitates, per cent of sulphur (SO_3) on samples as received and on dry

matter. Please report results as early as possible, and give the referee the benefit of your suggestions in regard to the work.

Compare the two following methods for total sulphur, using the cotton-seed meal and cowpeas:

SULPHUR.

Method I.—Place 5 grams material in a 2½-inch porcelain evaporating dish, add 20 cc concentrated nitric acid, and heat the mixture cautiously on the water bath until all danger of overflowing has passed. Then evaporate almost to dryness, add 10 cc of a 5 per cent solution of potassium nitrate, evaporate to complete dryness, and ignite, at first gently, then more vigorously, until the residue is white. The residue is then dissolved in hydrochloric acid and evaporated to dryness, and heated for some time in an air bath to render silica insoluble. Take up in water with the addition of a little hydrochloric acid, filter to 150 cc or more, precipitate the sulphuric acid with a solution of 1 gram barium chlorid, and complete the determination in the usual way. Make a blank test with the reagents.

Method II.—Fifteen grams of sodium peroxid are weighed and most of it introduced at once into a silver or nickel crucible and converted into hydroxid by adding a little water and boiling, preferably over an alcohol lamp, until the excess of water is expelled. The hydroxid is allowed to cool until pasty, when 2 grams of the material is stirred in it as quickly as possible. The heat is then raised and the remainder of the peroxid added in small portions to complete the oxidation. The fused mass, after cooling, is dissolved in water, neutralized with hydrochloric acid, evaporated to dryness, and heated in an air bath for some time to render silica insoluble. The residue is then taken up in water, with the addition of a little hydrochloric acid, filtered and washed to about 400 cc, and the sulphuric acid precipitated as before. Make a blank test with the reagents.

SULPHATES.

Determine the sulphates in the sample of sorghum by the following method:

Five grams of substance are mixed well with 50 cc of a 1 per cent solution of hydrochloric acid, allowed to stand half an hour, filtered, and washed with the dilute acid to 250 cc or more. The liquid is heated to boiling, barium chlorid added, and the determination completed in the usual way.

MOISTURE.

The results on moisture are stated as a matter of record.

TABLE I.—*Moisture determinations.*

Analyst.	No. 1. Cotton-seed meal.	No. 2. Cowpeas.	No. 3. Sorghum.
	Per cent.	*Per cent.*	*Per cent.*
F. P. Veitch, Washington, D. C.	5.98	8.55	7.75
Frank T. Shutt, Ottawa, Canada	5.90	8.43	7.14
	6.00	8.32	7.21
R. W. Thatcher, Washington (State)	5.35	7.90	6.36
	5.20	7.74	6.20
W. G. Morrison, North Carolina	6.28	8.59	.....
G. S. Fraps, Texas	5.89	8.28	.....

REMARKS OF ANALYSTS.

R. W. Thatcher.—Moisture was determined by drying seven hours in a boiling-water oven. It is my practice to dry in hydrogen, but appliances for this work were not available at the time this work was done.

G. S. Fraps.—The moisture determinations were made by drying eight hours on a watch glass at 100° C. It is my observation that drying five hours in a current of hydrogen gives usually higher results than by drying on a watch glass.

Total Sulphur.

The percentages of sulphur obtained by the methods to be compared are presented in the following table:

Table II.—*Determination of sulphur as SO_3.*

Analyst.	Cowpeas.		Cotton-seed meal.	
	Nitric-acid method.	Peroxid method.	Nitric-acid method.	Peroxid method.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*
F. P. Veitch, Washington, D. C.	0.466	0.539	1.071	1.133
Frank T. Shutt, Ottawa, Canada	.490	.....	1.115	.....
	.500	.....	1.098	.....
R. W. Thatcher, Washington (State)	.371	.....	.944	.....
	.379	.....	.953	.....
W. G. Morrison, North Carolina	.....	.583	.....	1.170
	.....	.593	.....	1.013
G. S. Fraps, Texas	.524	.501	1.120	1.129
	.532	.515	.....	1.230
Average of all determinations	.466	.544	1.048	1.135

Remarks of analysts.

F. P. Veitch.—The nitric-acid method has given in my hands results nearly as high as the peroxid method. The method was modified somewhat with a view to hastening the destruction of the organic matter. After adding potassium nitrate and burning some time the dish was cooled and a few cubic centimeters of nitric acid added, dried rapidly, and ignited cautiously. This gave a perfectly white ash and was much more rapid than the prescribed procedure. As much organic matter remains after the treatment with nitric acid, repeated treatment with nitric acid or aqua regia might give higher results.

In the peroxid method the silver crucible was strongly attacked in fusing sample No. 1. Some silver remained in solution even after evaporation and filtration. It was partly precipitated by dilution, but some remained in solution and was precipitated with the barium sulphate, which was purified by fusion with sodium carbonate, washed with water, dissolved in nitric acid, and reprecipitated with sulphuric acid. The precipitate in sample No. 2 was not contaminated by silver. In the present form I regard the peroxid method as the more accurate of the two methods. The correction for the sulphur in the peroxid was subtracted before reporting the results.

R. W. Thatcher.—I attempted to make the estimations of sulphur by the peroxid method, but only succeeded in driving everybody out of my end of the building, since our hoods are not yet completed sufficiently to carry off the fumes. The method seems to me to be a very undesirable one. In the first place, my sodium peroxid contains sulphur, and it is almost impossible to control the amount of sulphur by blank determinations since some sputtering of the material when water is first added is almost unavoidable. This fact along with the extreme difficulty of manipulation makes the method a very unsatisfactory one in my opinion.

W. G. Morrison.—The peroxid method was followed, nickel crucibles being used. The action was violent and there was an inclination toward spattering when sodium peroxid was added, and nickel appears every time in the filtrate on first filtering.

G. S. Fraps.—In the peroxid method a nickel or silver crucible of about 40 cc capacity gives more satisfactory results than a silver dish. Nickel is preferable,

since silver was dissolved in every determination made by the writer. The silver chlorid settled out when the liquid was diluted and allowed to stand, and it then could be filtered off. If the organic material is added to the sodium hydroxid before the water has been completely driven off, the mixture is very likely to foam over, and considerable foaming takes place in any event. It is therefore advisable to continue heating the peroxid for a few minutes after the water has apparently been driven off, then stir the organic matter in quickly, and heat cautiously until danger of foaming has passed.

A blank determination is always necessary, even with chemically pure peroxid. At its best the fusion method is very slow. It is possible that an iron crucible could be used in place of a silver or nickel one.

CONCLUSIONS.

There is no doubt in the mind of the referee that the nitric-acid method gives results slightly lower than the peroxid method. It is possible that the former may be so modified as to give higher results. Owing to the difficulty of manipulating the peroxid method and the disinclination of some chemists to work with it, the referee believes that it should be subjected to further study before adoption as a provisional method in place of the nitric-acid method.

DETERMINATION OF SULPHATES.

The referee had some difficulty in obtaining a sample suitable for a study of the method of determining sulphates in plants. None of the materials available seemed to contain appreciable quantities of sulphates. The referee last year reported on the determination of sulphates in a few samples, all of which were found to contain an exceedingly small proportion of inorganic sulphur. Sorghum and teosinte were examined and gave no visible precipitate of barium sulphate. Cotton seed were caused to germinate, but the germinated seed contained only 0.064 per cent of sulphates, and this method of preparing the samples did not appear promising.

The sample for analysis was prepared as follows: One gram of potassium acid sulphate was dissolved in 100 cc water, mixed thoroughly with 200 grams of sorghum until the sorghum was all damp, sifted, dried, and again sifted several times. It is believed that a uniform sample was obtained by this method.

The method for sulphates has been described. It is the method described in the report of the referee last year. The results of the different analysts are presented in the following table:

TABLE III.—*Determination of sulphates.*

Analyst.	Per cent.
F. P. Veitch, Washington, D. C	0.400
Frank T. Shutt, Ottawa	.375
	.376
R. W. Thatcher, Washington (State)	.349
	.324
G. S. Fraps, Texas	.379
	.381
	.380
	.367

CONCLUSIONS.

The method seems to give fairly satisfactory results in the hands of the different analysts, and while the amount of sulphates in agricultural products seems to be, in general, very small, it may be well to have a method for their determination.

RECOMMENDATIONS.

The referee makes the following recommendations:

(1) That the method for the determination of sulphates, as already described, be printed as a provisional method.

(2) That the peroxid method of determining sulphur be subjected to further study, with a view to substituting it for the nitric acid method.

The PRESIDENT. Is there any discussion of the paper presented, or of the subject of ash?

Mr. SHUTT. As associate referee, I tried the sodium peroxid method, and wish to indorse what is said in regard to the unsuitability of that method for the determination of sulphur. It is disagreeable, sloppy, and incorrect, and it does not seem at all likely that we shall ever be able to make a good working method of it. I scarcely think it will be of any value to continue this investigation. The whole method is one that seems to me quite unsuitable for good and accurate work.

Mr. PATRICK. If it were true that it were impossible to obtain sodium peroxid free from sulphur, I should agree with the view that it is hardly worth while to continue these experiments; but we have at the Bureau of Chemistry quite a quantity of this reagent entirely free from sulphur, and we are doing very good work with the process. I admit all that has been said concerning the troublesomeness and disagreeableness of the method.

The PRESIDENT. In the absence of the referee on tannin the report on insecticides will be received.

REPORT ON INSECTICIDES AND FUNGICIDES.

By J. K. HAYWOOD, *Referee.*

The work for this year upon insecticides and fungicides consisted of the testing of various methods outlined in previous years, together with a discussion of new methods published since the last report of the referee.

Samples of Paris green, London purple, copper carbonate, potassium cyanid, soda lye, soap, tobacco extract, and formalin were forwarded early in the spring to those who expressed a desire to cooperate in the work. The methods of analysis used were, in the main, the same as those of last year, with the addition of such changes as previous work had shown to be advisable. A new method for determining formaldehyde was also added, and the gravimetric hexa-methyl-tetramine method, which had been shown to be practically worthless, was dropped.

Results on Paris green were received from eight chemists, including the referee; on London purple from eight chemists; on copper carbonate from four chemists; on potassium cyanid from four chemists; on soda lye from three chemists; on tobacco extract from two chemists; and on formalin from three chemists.

Paris Green.

Following are the results obtained upon Paris green and the comments of the various analysts:

Table I.—*Paris green.*

Analyst.	Moisture.	Total arsenious oxid.		Total copper oxid.			Soluble arsenious oxid.	
		Method I.	Method II.	Method I.	Method II.	Electrolytic.	Method I.	Method II.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
James Emery, Biochemic Div., U. S. Dept. Agr.	1.00	60.00	53.30	27.74	30.67	.....	7.04	.....
	1.02	60.20	53.62	26.93	30.55	.....	6.14	.....
J. M. Price, Biochemic Div., U. S. Dept. Agr	.....	.....	53.77	27.53	30.64	.....	7.06	.....
	.....	.....	53.77	27.60	.....	.....	7.06	.....
R. W. Thatcher, Pullman, Wash	.56	60.04	53.00	29.38	29.14	.....	7.09	7.88
	.55	59.96	55.65	29.55	29.25	.....	7.03	7.73
H. J. Warner, Bur. of Chem., U. S. Dept. Agr	.....	60.15	55.44	28.31	28.61	.....	7.52	9.85
	.....	60.25	55.69	28.14	28.77	.....	7.42	9.85
R. J. Davidson and J. B. McBryde, Blacksburg, Va	.54	.....	53.49	28.84	29.18	28.78	6.83	6.03
	.53	.....	53.64	28.74	29.10	28.97	6.82	5.97
	.....	.....	53.66	28.88	29.26	28.90	.....	.....
	.....	.....	53.53	28.87	.....	.....	.....	.....
A. G. Ford, Stillwater, Okla	.85	59.61	53.39	28.03	27.81	.....	7.21	.....
	1.00	59.86	53.49	28.03	28.20	.....	7.50	.....
	.....	59.61	53.25	28.28	28.29	.....	7.21	.....
	.....	59.67	53.25	28.03	.....	.....	.....	.....
W. P. Allen, New Brunswick, N. J.	1.31	59.52	.....	27.72	27.93	.....	7.16	8.29
	1.25	59.58	.....	27.82	27.98	.....	7.17	8.29
	.....	.....	.....	27.84	28.08	.....	7.27	7.67
	.....	.....	.....	.....	28.09	.....	.....	.....
	.....	.....	.....	.....	28.21	.....	.....	.....
	.....	.....	.....	.....	28.26	.....	.....	.....
J. K. Haywood, Bur. of Chem., U. S. Dept. Agr.	.70	59.94	54.92	28.24	28.95	28.63	7.20	8.07
	.63	59.87	54.13	28.33	29.04	.....	7.12	8.31

COMMENTS BY ANALYSTS.

R. W. Thatcher.—In Method II for total arsenious oxid I stirred for some time in an attempt to dissolve all the free arsenious oxid but did not get all the crystals to dissolve, however. I then used a modification suggested by Avery, as follows: A weighed sample of the green was boiled with sodium acetate as in the determination of soluble arsenic by his method, the mixture filtered through asbestos, the soluble arsenic determined in the filtrate and the residue on the asbestos dissolved in dilute hydrochloric acid and titrated according to the Avery-Beans method. The total arsenic figures determined in this way were 59.90 and 59.84 per cent. I found in Method I for soluble arsenic that as the weight of green taken varied, the soluble arsenic varied according to the following figures: One gram of green gives 7.09 and 7.03 per cent soluble arsenious oxid; 0.809 gram green gives 7.29 per cent soluble arsenious oxid; 0.484 gram green gives 7.51 per cent soluble arsenious oxid.

R. J. Davidson and *J. B. McBryde.*—In method II for total arsenious oxid varying results were obtained, according to the amount of hydrochloric acid added, and the time of stirring. The results sent in by us were obtained by using 1 cc of hydrochloric acid to each determination and stirring frequently for 10 minutes; an N/20 iodin solution was used.

In method I for total copper oxid the results were obtained by using 3 grams of potassium iodid to each determination. With only ten times the weight of copper, it was found impossible to obtain concordant results. An N/20 thio solution was used throughout.

In method II for total copper oxid N/10 potassium cyanid was used.

A. G. Ford.—In method II for total arsenious oxid a white residue remained, which did not go into solution, even on the addition of 15 drops of hydrochloric acid and 24 hours' standing. I therefore filtered off the solution and washed the residue with cold water; placed the filter paper in a 250 cc flask (along with blank determination), digested 10 minutes with 25 cc of 2 per cent sodium hydrate, added 25 cc of hydrochloric acid and 3 grams of potassium iodid after cooling to 80°, and allowed to stand 15 minutes; diluted, decolorized with thio sulphate, made alkaline with soda, then acid with hydrochloric acid, and finally added excess of sodium bicarbonate and titrated with standard iodin. By adding the figure thus obtained to the previous figures the result was 59.93 per cent arsenious oxid.

W. P. Allen.—In method II for total copper oxid the potassium cyanid solution must be added very slowly or more will be used than is necessary.

J. K. Haywood.—Method II for total arsenious oxid, as would be expected from last year's work, gave low results. I would suggest either of the following modifications of this method; (*a*) Pulverize the sample and weigh out about 0.3 to 0.4 grams in a beaker, add 25 cc of water and, while constantly stirring, add concentrated hydrochloric acid a drop at a time until all Paris green is in solution and free arsenious oxid remains as a residue. Filter and wash the residue and determine the arsenious oxid in the filtrate according to the regular Avery-Beans method. The filter and contents are washed back into the beaker, 5 grams of sodium bicarbonate added, and the solution boiled until all arsenious oxid has dissolved. The resulting solution is cooled, slightly acidified with hydrochloric acid, and made alkaline again with sodium bicarbonate and finally titrated with iodin. (*b*) Take 0.4 gram of the finely ground green and boil with 25 cc of sodium acetate (containing 12 grams) for 10 minutes. Then add concentrated hydrochloric acid a drop at a time until solution is effected (about 10 cc of the acid will be necessary). Add concentrated sodium carbonate solution a drop at a time until a slight precipitate appears, then add a solution containing 2 to 3 grams of sodium potassium tartrate and finally sodium bicarbonate in excess. Titrate with iodin in the usual manner. By method (*a*) 59.87 per cent of total arsenious oxid was obtained and by method (*b*) 59.62 per cent.

Method I for total copper oxid appears to give results somewhat lower than the truth and method II somewhat higher results.

DISCUSSION OF THE RESULTS.

It will be at once seen that method I for total arsenious oxid has given most excellent results, as it did last year. Method II has given low and variable results, as was also to be expected from previous work. There is no doubt that method II would prove of great value if modified to include free arsenious oxid. Your referee has therefore spent some time working upon this method and suggests the above modifications, which give most excellent results. Others have evidently seen the advisability of changing this method, as in the comments of the analysts given above two other modifications are suggested, one by R. W. Thatcher which was originally

suggested to him by Mr. Avery, and one by A. G. Ford. Both of these modifications evidently give good results.

Just after the method above, marked (*a*), was sent to press your referee entered into correspondence with Mr. Avery, one of the originators of the method, and found that he was working along the same line and had also sent a modification of his method to the publishers. Mr. Avery was kind enough to forward two modifications that he had worked out. They are as follows:

(*c*) Weigh out a small quantity of the finely ground green and treat with N/2 hydrochloric acid, from 5 to 10 cc for each 0.1 gram of the green present. Boil gently; if solution is not effected add a cold saturated solution of sodium acetate, using about 3 grams for each 0.1 gram of the green, and boil until all arsenious oxid is dissolved. Now add alkaline tartrate and solid bicarbonate and after dilution titrate in the ordinary way.

(*d*) Use the method as given above by Mr. Thatcher, which was originally suggested by Mr. Avery.

Your referee tested both of these methods and found that they gave most excellent results—60.30 per cent by the former and 60.04 per cent by the latter. After receiving the two methods from Mr. Avery the referee then thought of the other modifications marked (*b*) above. Since the modifications proposed by Mr. Avery were of great value to him in working out method (*b*), he feels that this method is really a joint production with Mr. Avery rather than any original idea of his own.

It will thus be seen that four modifications are given above, each of which gives excellent results. The one to be used would best be determined by the exigencies of the case.

While the results obtained with method I for total copper oxid by any one analyst agree with each other very well, the averages of the results obtained by different chemists vary quite a good deal, and this seems to be true also for method II. Judging from all the results there appears to be a marked tendency in the direction of low results for method I and high results by method II. There are a few exceptions to this—one in the case of Mr. Thatcher, who obtains higher results by method I than by method II, and one in the case of Mr. Ford, who obtained practically the same figures by each method. Since it is only by averages, however, that one can judge, the referee is inclined to believe that it would be best to adopt the electrolytic as the official method and allow the others for the present to remain as provisional, to be used by those who obtain as good results with them as with the electrolytic method.

A comparison of the figures obtained by the individual analysts and the averages given by the various chemists using method I for soluble arsenious oxid shows a fair agreement when the difficulty of separating the free from the combined arsenious oxid is considered. The results obtained by method II for soluble arsenious oxid are in the main higher than those obtained by method I. This is what would be expected from previous work. The agreement between these results leaves a good deal to be desired and plainly shows that this method, which at the best is only approximate, must be carried out under rigid rules in order to get reliable results.

London Purple.

Table II.—*London purple.*

Analyst.	Moisture.	Total arsenious oxid.	Total arsenic oxid.	Soluble arsenious oxid.	Soluble arsenic oxid.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
James Emery, Biochemic Div., U. S. Dept. Agr	.....	12.61	30.59	.....	.....
	.....	12.68	.....	.....	.....
J. M. Price, Biochemic Div., U. S. Dept. Agr	.....	12.98	29.79	.....	.....
R. W. Thatcher, Pullman, Wash	0.95	12.65	29.78	3.61	9.37
	.93	12.63	30.00	3.61	9.30
H. J. Warner, Bur. of Chem., U. S. Dept. Agr	.....	12.92	30.19	2.58	6.67
	.....	.....	.....	2.58	6.53
	.....	.....	.....	2.81	6.67
R. J. Davidson and J. B. McBryde, Blacksburg, Va.	2.12	12.77	29.68	2.48	6.66
	2.19	12.67	29.74	2.48	6.61
	.....	.....	.....	2.48	6.72
	.....	.....	.....	2.48	6.69
A. G. Ford, Stillwater, Okla	1.71	12.72	32.26	3.04	4.62
	1.71	12.72	.....	3.04	.....
W. P. Allen, New Brunswick, N. J	2.39	11.62	31.56	1.62	.....
	2.41	11.71	31.56	1.85	.....
	.....	11.75	31.56	1.97	.....
J. K. Haywood, Bur. of Chem., U. S. Dept. Agr	2.44	13.03	29.70	2.37	6.27
	2.26	13.03	29.70	2.61	6.67
	.....	.....	.....	2.61	6.34

Comments by analysts.

R. J. Davidson and J. B. McBryde.—In making all iodin titrations N/20 iodin was used. In determining the total arsenic oxid it is very difficult to read the end point with thio-sulphate. We therefore suggest that some method of getting rid of part of the coloring matter could be used to advantage. One way would be to use 200 cc of the solution and proceed as under total arsenious oxid until the solution is filtered off, then acidify a portion with hydrochloric acid at 80° and proceed as under arsenic oxid. Another way would be to use 100 cc instead of 50 cc, as under total arsenic oxid, make alkaline with sodium carbonate and make up to a definite volume. Filter off half of the solution, acidify with hydrochloric acid, heat to 80°, and proceed as under total arsenic oxid.

A. G. Ford.—In determining total and soluble arsenic oxid I found it extremely difficult to read the end reactions with thio-sulphate and iodin within a range of 0.3 to 0.5 cc.

Discussion of results.

A glance at column 2 shows that the results obtained for total arsenious oxid in London purple agree with each other extremely well, with possibly one exception.

The results obtained by six different chemists upon total arsenic oxid agree very well with each other, while two chemists obtained high results.

Your referee is loath to drop this method of examining London purple since it is the only method known to him for determining both forms of arsenic in this material, and, moreover, the figures for this and previous years show that a majority of the chemists using it get very reliable results. The referee also knows from a personal experience of two or three years that when one is familiar with the method very accurate results can be obtained. It is of course difficult to read the end points without previous experience, therefore any modification of this method which

would so far rid the solution of coloring matter as to make the readings easy, even for the inexperienced, would be of great value. Two such modifications have been suggested by Messrs. Davidson and McBryde in their comments given above. The referee is therefore of the opinion that no better work could be performed during the coming year by the referee on insecticides and fungicides than a testing of the modifications given by Messrs. Davidson and McBryde and such other modifications as might suggest themselves.

The results obtained for soluble arsenious oxid by four chemists agree fairly well with each other. One chemist obtains slightly low results and one high results. The figures for soluble arsenic oxid show an excellent agreement in three cases, while in the other two cases they vary considerably. These two methods of examination also can only lead to reliable results by following very strict rules of procedure, just as in the determination of crude fiber. It has been the experience of the referee that results within about 0.25 to 0.75 per cent of each other (according to the amount of soluble arsenious and arsenic oxid present) can be obtained by these two methods, which are exact enough for all practical purposes, although more correct figures are greatly to be desired for scientific purposes. It might be well for someone to undertake work to determine whether a shorter time of standing would be long enough for all of the soluble arsenious and arsenic oxids to go into solution and whether more concordant results would not be obtained by a shorter period of standing.

Copper Carbonate.

Table III.—*Copper carbonate.*

Analyst.	Total copper oxid.		
	Method I.	Method II.	Electrolytic method.
	Per cent.	*Per cent.*	*Per cent.*
H. J. Warner, Bur. of Chem., U. S. Dept. Agr	59.50	59.25	.....
	59.50	59.25	.....
R. J. Davidson and J. B. McBryde, Blacksburg, Va	59.12	58.42	58.31
	58.88	58.22	.....
A. G. Ford, Stillwater, Okla	58.57	58.71	.....
	58.77	59.10	.....
	58.06	58.71	.....
	58.57	58.32	.....
J. K. Haywood, Bur. of Chem., U. S. Dept. Agr	58.11	59.67	58.73
	58.48	59.67	.....

Comments by Analysts.

R. J. Davidson and J. B. McBryde.—In method I N/20 thio-sulphate was used, but even with this strength a difference of reading of 0.05 cc gave a difference of more than 0.2 per cent in the results. In method II N/10 potassium-cyanid solution was used, but, as in the case of thio, a difference of 0.1 cc gave a difference of 0.2 per cent in the results.

J. K. Haywood.—As in the determination of total copper in paris green I obtained slightly low results by the thio-sulphate method and high results by the potassium-cyanid method.

Discussion of the Results.

A discussion of these figures is rather difficult, as no general law, such as was shown by the figures for total copper in Paris green, is brought out. One chemist appears

to get slightly high results by both methods, one to get high results by method I and correct results by method II, one to get equally correct results by both methods and one to get slightly low results by method I and extremely high results by method II. It would therefore appear that, as in the case of Paris green, it would be best to adopt the electrolytic as the official method and leave the other two methods as provisional, to be followed or not, according as the individual chemist deems it advisable upon comparing the results so obtained by him with those given by the standard electrolytic method. From the remarks of Messrs. Davidson and McBryde, it appears extremely likely that a greater part of the variation in the above figures was caused by too great a strength of the standard solution. A valuable piece of work would be performed if the referee for the coming year could work out just what the factors are causing the slight variation in the results obtained by different chemists.

Potassium Cyanid.

Table IV.—*Potassium cyanid.*

Analyst.	Cyanogen.		Chlorin.
	Volumetric.	Gravimetric.	
	Per cent.	*Per cent.*	*Per cent.*
James Emery, Biochemic Div., U. S. Dept. Agr.	39.42	.....	8.83
	39.32	.....	.....
T. M. Price, Biochemic Div., U. S. Dept. Agr.	39.32	.....	.....
H. J. Warner, Bur. of Chem., U. S. Dept. Agr.	39.46	.....	.....
	39.60	.....	.....
R. J. Davidson and J. B. McBryde, Blacksburg, Va.	39.90	.....	.....
	39.78	.....	.....
J. K. Haywood, Bur. of Chem., U. S. Dept. Agr.	40.49	40.42	8.98
	40.49	.....	.....
	40.11	.....	.....

COMMENT BY ANALYSTS.

R. J. Davidson and J. B. McBryde.—Two separate portions were weighed out for analysis, and the titrations of the two samples agree within 0.05 cc. The difference in percentage is 0.3 per cent. In other words, one drop of N/10 silver solution is equal to 0.3 per cent potassium cyanid. Duplicate titrations with N/10 iodin gave 39.80 per cent of cyanogen.

J. K. Haywood.—I first determined the cyanogen according to directions. This method is based on the following equation:

$$2KCN+AgNO_3=KCNAgCN+KNO_3.$$

In other words, we might say that only one-half of the potassium cyanid is titrated. I then added potassium chromate and continued the titration until the appearance of the usual brown color of silver chromate. This last titration not only gives the other half of the cyanogen according to the equation $KCNAgCN+AgNO_3=KNO_3+2\,AgCN$, but gives any chlorin that may be present as silver chlorid. Therefore, if we represent the number of cubic centimeters used in the first titration by M and the number used in the second titration by N, then N−M=the number of cubic centimeters used by chlorin, and 2 M is the number used by the cyanogen according to the following equation:

$$2KCN+2AgNO_3=2KNO_3+2AgCN.$$

Having thus determined the chlorin, the total amount of silver chlorid and silver cyanid was determined gravimetrically and from this was subtracted the amount of

silver chlorid corresponding to the chlorin figure, thus leaving the silver cyanid, from which the result 40.42 per cent of cyanogen was obtained.

DISCUSSION OF THE RESULTS.

The referee sent out this sample thinking that it was a pure sample of potassium cyanid, but found out soon after that it was a mixture of sodium and potassium cyanids, sodium chlorid, and other ingredients in such proportions that just about the amount of cyanogen was present as would be present in chemically pure potassium cyanid. This explains the chlorin figures given by both the referee and the associate referee. Of course the presence of this chlorin would not interfere in any way with the volumetric determination of the cyanogen present. The results obtained show excellent agreement, with the possible exception of the referee's figures, which are rather high. This is undoubtedly due to the fact that the referee worked upon the sample when it was first put up, while the other chemists worked upon it some months later, when the cyanid, which is very hygroscopic, had gained in moisture. On account of the excellent agreement of the results obtained during the last two years' work, and also because the gravimetric determination shows that the volumetric is correct, it would seem that this method ought to be adopted as one of the official methods, with one change, however, and that is, an N/20 instead of an N/10 silver nitrate solution should be used.

Soda Lye.

TABLE V.—*Soda lye.*

Analyst.	Sodium hydroxid.		Sodium carbonate.	
	Method I.	Method II.	Method I.	Method II.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
H. J. Warner, Bur. of Chem., U. S. Dept. Agr	73.44	73.16	4.47	4.60
	73.44	73.16	4.47	4.60
R. J. Davidson and J. B. McBryde, Blacksburg, Va	73.98	71.57	3.91	5.53
	73.58	71.57	4.15	4.96
J. K. Haywood, Bur. of Chem., U. S. Dept. Agr	73.59	72.87	4.20	4.13
	73.59	72.87	4.20	4.13

COMMENTS BY ANALYSTS.

R. J. Davidson and J. B. McBryde.—Under method I for sodium hydroxid, tests were made with the portion to which barium chlorid was added, in order to determine the possibility of titrating the solution without removing the precipitate of barium carbonate. As soon as barium chlorid was added to the solution samples were drawn off and titrated. The results obtained in this way were identical with those obtained from decanted and filtered samples. Our tests go to show that the removal of the barium carbonate from the solution is unnecessary.

We also made tests to determine the possibility of using only one indicator under method I. After the addition of barium chlorid, methyl orange gave results identical with those obtained when using phenolphthalein. By using phenolphthalein in boiling solution we obtained the same results in total alkalies as when using methyl orange.

DISCUSSION OF RESULTS.

A consideration of this table shows that all of the chemists obtained closely agreeing results on sodium hydroxid, and very fair results on sodium carbonate, by method I. The results on these two constituents by method II do not agree nearly so well. Just as in last year's work, it will be noted that all of the analysts obtained higher

figures for sodium hydrate by method I than by method II, and lower results for sodium carbonate by method I than by method II. Your referee gave last year his reasons for believing that the results obtained by method II were more accurate than those obtained by method I, but it does not appear from either year's work that method II is as easy of execution as method I. It is suggested that the only way of telling which is the better method is by making an actual gravimetric determination of the carbon dioxid present. This should be done during the coming year, so that method I, which now appears to give the most concordant results, may either be adopted as an official method or rejected. In case method I is found to give low results on sodium carbonate, method II could be further worked upon to find the cause of the variations in results by different chemists. It would appear from the work of Messrs. Davidson and McBryde that a removal of the precipitated barium carbonate in method I is not necessary. This, if true, is a very valuable point, in that it greatly shortens the time necessary for making a determination.

Tobacco Extract.

Table VI.—*Tobacco extract.*

Analyst.	Nicotin.	
	Method I.	Method II.
	Per cent.	*Per cent.*
R. J. Davidson and J. B. McBryde, Blacksburg, Va	22.37	22.92
	22.64	22.69
	23.08	21.94
	.....	21.39
J. K. Haywood, Bur. of Chem., U. S. Dept. Agr	23.53	21.93
	23.70	21.88
	22.70	21.74

Comments by analysts.

R. G. Davidson and J. B. McBryde.—In method II the substance was weighed out in small beakers and allowed to stand, one 24 hours, one 36 hours, and two for 48 hours.

J. K. Haywood.—In both methods I and II it was found necessary to distill over about 500 cc of water in order to obtain all the nicotin. In method II an attempt was made to drive off all ammonia by heating to 50°–60° C. after adding the sodium hydroxid, but a great loss of nicotin also took place. It was also found that the longer the extract was allowed to stand in the open in contact with sodium hydrate, the smaller the figure obtained for nicotin, showing that there was a constant loss of this substance.

Discussion of the results.

From the work done last year it was evident that the Lloyd method gave low results upon extracts containing a large amount of nicotin. It was also found that the Winton method (II) gave extremely high results in case large amounts of ammonia and ammonium salts were present, unless the sodium hydroxid was allowed to stand in contact with the extract for a very long period. This year's results show that if large quantities of ammonium salts and ammonia are not present fair results can be obtained by method II, but there is a continuous loss of nicotin the longer the extract stands in the open in contact with the sodium hydrate. It is manifestly impossible to tell with all the extracts the exact point at which all the ammonia is given off and no nicotin has yet volatilized. In fact, such a point very

likely does not exist. It would therefore appear that both the Lloyd method and method II must be rejected, which leaves only method I. Last year very fair results were obtained by this method, considering the difficulty of the determination, and the experience has been repeated this year. Since this is so, and the Kissling method has stood the test of time when used by various chemists outside of the association, your referee is of the opinion that it should be adopted as the official method of the association, with an understanding, however, of its limitations, the principal of which is that a large part of the pyridin present is determined as nicotin.

Formalin.

Table VII.—*Formalin.*

Analyst.	Formaldehyde.		
	Method I.	Method II.	Method III.
	Per cent.	*Per cent.*	*Per cent.*
J. K. Haywood, Bur. of Chem., U. S. Dept. Agr	18.82	18.96	18.58
	18.96	18.81	18.68
B. H. Smith, Bur. of Chem., U. S. Dept. Agr	18.86	18.80	18.40
	18.93	18.86	18.16
	18.92	.....	.....
F. F. Ladd and C. H. Kimberly, Agricultural College, N. Dak	22.4	21.0	18.99
	19.4	21.0	18.94
	22.0	18.0	19.08

COMMENTS BY ANALYSTS.

Messrs. Ladd and Kimberly.—Method I gives varying results according to the length of time the sample stands. We believe the results to be high. Method II seems open to serious objections, since a slight error in reading causes a large error. A difference of 0.1 cc may make an error of 3 per cent in strength. Method III has always given the most trustworthy results for laboratory use, and rosolic acid is a more satisfactory indicator than litmus. No corrections have been made for free acids present.

DISCUSSION OF THE RESULTS.

From the table above it appears that two chemists were able to get most excellent comparative results by both methods I and II, while a third chemist obtained widely varying results by these two methods. For method III the first two chemists obtained low results, while the third chemist obtained very accurate results. The referee can not agree with Messrs. Ladd and Kimberly in their conclusion that method I gives unreliable, and method III extremely reliable, results. It has been his experience that method I gives very excellent results, while method III always gives low results, and is further open to the objection that it is a very hard matter to read the end point. It is perfectly true that method II is open to the objection that a very slight error in the reading causes a large error in the percentage, and it would appear that method II should only be used upon very dilute solutions of formaldehyde.

It might be well to say just here that method II is not the same as method II of last year, which was replaced by the present method proposed by Romijn,[a] and is as follows:

Fifteen cubic centimeters of N/10 silver nitrate are treated with six drops of 50 per cent nitric acid in a 50 cc flask; 10 cc of a solution of potassium cyanid (containing 3.1 grams of potassium cyanid in 500 cc of water) are then added and well shaken.

[a] Zeit. anal. Chem., 1897, **36**: 18–24.

An aliquot portion of this filtrate, say 25 cc, is titrated according to the method of Volhard with an N/10 solution of ammonium-sulpho-cyanate for excess of silver. Another 15 cc portion of N/10 silver nitrate is acidified with 50 per cent nitric acid and treated with 10 cc of the potassium cyanid solution to which has been added a weighed quantity of the dilute formaldehyde solution. The whole is made up to 50 cc and a 25 cc filtrate from it is titrated with N/10 ammonium-sulpho-cyanate for the excess of silver as before. The difference between these results multiplied by 2 gives the amount of potassium cyanid that has been used by the formaldehyde in terms of N/10 ammonium-sulpho-cyanate. Each cubic centimeter of this is equal to 3 mg of formaldehyde.

Since this difference of opinion exists between your referee and two of the chemists who were engaged in the work, it would appear advisable to examine the opinions that have been published on the subject.

C. Wallnitz, in an article[a] which appeared early in 1903, compared several methods for determining formaldehyde. He found that the hydrogen peroxid (method I) and the iodin methods were superior to the others tested when the reagents are freshly prepared, and that the Legler method (method III) lacked distinctness in the end point reading.

B. H. Smith, in an article[b] published during the fall of 1903, found that the hydrogen peroxid method (method I) is very satisfactory for strong solutions; that the Legler method (method III), while not giving quite as high results, is fairly satisfactory, and that the potassium cyanid method (method II) gives good results on dilute solutions.

The referee will, therefore, make no recommendations concerning the adoption of any of these methods, but will leave them until the work of the association definitely establishes the superiority of one or the other.

Recommendations.

I would respectfully recommend:

(1) That method I for total arsenious oxid in Paris green be adopted as an official method, this being a repetition of my last year's recommendation.

(2) That the four modifications of the Avery-Bean method for total arsenic in Paris green be tested to find which is the best modification to be adopted as an optional official method.

(3) That the electrolytic method be adopted as the official method for determining copper in Paris green and in copper carbonate, and that the volumetric methods be further tested, paying particular attention to the remarks of Messrs. Davidson, McBryde, and Allen upon this subject.

(4) That an attempt be made to so change the procedure in the analysis of London purple that some of the organic matter may be removed. The remarks of Messrs. Davidson and McBryde are of value in this connection.

(5) That the volumetric silver nitrate method be adopted as the official method of determining cyanogen in potassium cyanid and that an N/20 instead of an N/10 solution of silver nitrate be used.

(6) That the methods of examination of soda lye be further tested and compared with the gravimetric methods and that points brought out by Messrs. Davidson and McBryde be given consideration.

(7) That the Kissling method for determining nicotin be adopted as an official method.

(8) That especial attention be given to the methods for determining formaldehyde in order to avoid needless repetition of work.

I desire to express my thanks to those gentlemen who have so kindly and ably cooperated with me in the above work, and especially to Messrs. Davidson and McBryde for many valuable suggestions.

[a] Zeit. angew. Chem., April 7, 1903. [b] J. Am. Chem. Soc., 1903, vol. 25, No. 10.

(A discussion by Messrs. Davidson, Mewborne, Hird, and Haywood on the subject of nicotin determinations, especially in strong extracts, followed the reading of the report. The points discussed are brought out in the report as printed in full.)

The PRESIDENT. In the absence of the referee on tannin the report of the committee on fertilizer legislation will be received.

REPORT OF COMMITTEE ON FERTILIZER LEGISLATION.

By H. W. WILEY, *Chairman.*

As it was not practicable to call a meeting of the committee on fertilizer legislation, I corresponded with each member of the committee to secure an expression of opinion, first, as to the necessity of legislation regulating interstate commerce and foreign commerce in fertilizers and fertilizer ingredients; and, second, in regard to the character that such legislation should assume. I have received replies from members of the committee, and present the correspondence for publication.

WASHINGTON, D. C., *October 29, 1903.*

DEAR SIR: The necessity for some national legislation in regard to fertilizers is becoming more and more evident every day. The States, of course, have legislation controlling commerce wholly within the States, but their authority does not extend beyond the State line. The multiplicity of labels and regulations in the different States is a real annoyance to manufacturers and to the trade.

It is believed that national legislation controlling interstate commerce in fertilizers and commerce therein in the District of Columbia and the Territories and regulating foreign commerce therein would prove beneficial in many ways. Such legislation, if wisely drawn, would serve as a model in the different States and would gradually secure a greater uniformity in State laws, thus removing unnecessary restrictions on trade, both in manufacture and in transit.

The committee on fertilizer legislation of the Association of Official Agricultural Chemists should make some recommendations at the coming meeting respecting national legislation. The committee doubtless could also properly make suggestions in regard to uniform State legislation. I therefore ask that you express your opinion on the following points:

(1) Is national legislation of the kind indicated above desirable?

(2) If so, suggest tentatively the form of an act which should be brought before the Congress of the United States.

(3) State how, in your opinion, national legislation should influence State legislation in regard to uniformity and freedom from unnecessary restrictions on commerce.

(4) Miscellaneous matters respecting fertilizer legislation not included in the above items.

I shall be glad to have a full expression of your views on these points and earnestly request that you place this expression in my hands not later than November 14, proximo, in order that the chairman may have an opportunity to collate and present the views of the different members of the committee to the association.

Respectfully,

H. W. WILEY,
Chairman Committee on Fertilizer Legislation, A. O. A. C.

RALEIGH, N. C., *October 30, 1903.*

DEAR SIR: I have your favor of the 29th in regard to national fertilizer legislation. I think some legislation along this line is probably desirable. The only difficulty I see is that in case the bill went into details it might give trouble in some of the States. You will remember that we had considerable difficulty in agreeing on the names by which to designate the fertilizing constituents in our committee a few years ago. Some ten of the Southern States have practically agreed on a uniform law, which was framed by the State chemists and commissioners of agriculture in an association known as the Cotton States Association of Commissioners of Agriculture. A copy of this law is to be found on page 52 of the proceedings of this association, which I am

sending under separate cover.[a] In this association we had difficulty in agreeing on names by which to designate the constituents. The main features of this bill, that is, the portions relating to uniform branding and guaranties, is now in operation, or will be in operation in Virginia, North Carolina, Tennessee, Alabama, Georgia, Louisiana, and Arkansas, with prospects that Florida and South Carolina will conform to it. I think the details of inspection and the control of the fertilizer trade should be left to each individual State. While our bill includes all the minutiæ, it was understood in our discussion of it that each State was at liberty to arrange the minor details to suit conditions.

B. W. KILGORE, *State Chemist.*

FERTILIZER LAW DRAFTED BY COMMITTEE OF THE COTTON STATES ASSOCIATION OF COMMISSIONERS OF AGRICULTURE.

A BILL to be entitled an act to regulate and make uniform with the acts of other States of the Union the registration, sale, inspection, and analysis of commercial fertilizers, acid phosphates, fertilizer materials, and chemicals, in the State of ———, and to consolidate all laws relating to said sales, inspection, and analysis, and to repeal all other laws, or parts of laws, in conflict therewith.

SECTION 1. *Be it enacted by the general assembly of the State of* ———, That all manufacturers, jobbers, and manipulators of commercial fertilizers and fertilizer materials to be used in the manufacture of the same, who may desire to sell or offer for sale in the State ——— such fertilizers and fertilizer materials, shall first file with ———, of the State of ———, upon forms furnished by said ———, the name of each brand of fertilizer, acid phosphate, fertilizer material, or chemical which they may desire to sell in the said State, either by themselves or their agents, together with the name and address of the manufacturer or manipulator; and also the guaranteed analysis thereof, stating the sources from which the phosphoric acid, nitrogen, and potash are derived, and if the same fertilizer is sold under a different name or names, said fact shall be so stated, and the different brands which are identical shall be named.

SEC. 2. All persons, companies, manufacturers, dealers, or agents, before selling or offering for sale in this State any commercial fertilizer or fertilizer material, shall brand or attach to each bag, barrel, or package the brand name of the fertilizer, the weight of the package, the name and address of the manufacturer, and the guaranteed analysis of the fertilizer, giving the valuable constituents of the fertilizer in minimum percentages only. These items shall be branded or printed on the package in the following order:

1. Weight of each package in pounds.
2. Brand name or trade-mark.
3. Guaranteed analysis.
4. Available phosphoric acid.....per cent.
5. Nitrogen.....per cent.
6. Potash.....per cent.
7. Name and address of the manufacturer.

In bone meal, tankage, or other products, where the phosphoric acid is not available to laboratory methods, but becomes available on the decomposition of the products in the soil, the phosphoric acid shall be claimed as total phosphoric acid, unless it be desired to claim available phosphoric acid also, in which latter case the guaranty must take the form above set forth. In the case of bone meal and tankage, manufacturers may brand on the bags information showing the fineness of the product, provided it takes a form approved by the ———.

SEC. 3. If any commercial fertilizer or fertilizer material offered for sale in this State shall, upon official analysis, prove deficient in any of its ingredients, as guaranteed and branded upon the sacks or packages, and if by reason of such deficiency the commercial value thereof shall fall 3 per cent below the guaranteed total commercial value of such fertilizer or fertilizer material, then any note or obligation given in payment thereof shall be collectible by law only for the amount of the actual total commercial value as ascertained by said official analysis.

SEC. 4. Be it further enacted, that the words "High grade" shall not appear upon any bag or other package of any complete fertilizer which complete fertilizer contains, by its guaranteed analysis, less than 10 per cent available phosphoric acid, 1.65 per cent nitrogen (equivalent to 2 per cent of ammonia), and 2 per cent of potash, or a grade or analysis of equal total commercial value; that the word "Standard" shall

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[a] A copy of this law follows.

not appear upon any bag or other package of any complete fertilizer which contains, by its guaranteed analysis, less than 8 per cent available phosphoric acid, 1.65 per cent nitrogen (equivalent to 2 per cent ammonia), and 2 per cent potash, or a grade or analysis of equal total commercial value; that the words "High grade" shall not appear upon any bag or other package of any acid phosphate with potash which shall contain by its guaranteed analysis less than 13 per cent available phosphoric acid and 1 per cent of potash, or a grade or analysis of equal total commercial value; that the word "Standard" shall not appear upon any bag or other package of any acid phosphate with potash which shall contain by its guaranteed analysis less than 11 per cent available phosphoric acid and 1 per cent potash, or a grade or analysis of equal total commercial value; that the words "High grade" shall not appear upon any bag or other package of any plain acid phosphate which shall contain by its guaranteed analysis less than 14 per cent available phosphoric acid; and, lastly, that the word "Standard" shall not appear upon any bag or other package of any plain acid phosphate which shall contain, by its guaranteed analysis, less than 12 per cent available phosphoric acid.

It is further hereby provided that no complete fertilizer, acid phosphate with potash, acid phosphate with nitrogen, or plain acid phosphate shall be offered for sale in this State which contains less than 12 per cent of total plant food, namely, available phosphoric acid, nitrogen, and potash, either singly or in combination, provided that in mixed fertilizers there shall not be claimed less than 1 per cent of potash and 0.82 per cent of nitrogen when one or both are present in the same mixture.

Sec. 5. Be it further enacted, that all manufacturers and manipulators, or agents representing them, who have registered their brands in compliance with section 1 of of this act, shall forward to ——— a request for tax tags, stating that said tags are to be used upon brands of fertilizers and fertilizer materials registered in accordance with this act, and said request shall be accompanied with the sum of ——— cents per ton as an inspection fee, whereupon it shall be the duty of ——— to issue tags to parties applying, who shall attach a tag to each bag, barrel, or package thereof, which, when attached to said package, shall be prima facie evidence that the seller has complied with the requirements of this act. Any tags left in the possession of the manufacturers shall not be used for another season, but shall be redeemed with new tags within sixty days after the close of the department's fiscal year, which fiscal year shall be comprised between the dates ———, inclusive. The color of the said tax tags must be changed each fiscal year, and there shall not be printed upon said tax tags any brand name or analysis.

Sec. 6. Be it further enacted, That it shall not be lawful for any manufacturer or company, either by themselves or their agents, to sell or offer for sale in this State any fertilizer or fertilizer material that has not been registered with ———, as required by this act. The fact that the purchaser waives the inspection and analysis thereof shall be no protection to said party selling or offering the same for sale.

Sec. 7. The guaranteed analysis of each and every brand of fertilizer or fertilizer material must, without exception, remain uniform throughout the fiscal year for which it is registered, and in no case, even at subsequent registration, shall the grade be lowered, although the proportion of the available constituents may be changed so that the decrease of one constituent may be compensated for in value by the increase of the other or others. Such proposed change must first receive the approval of the ———. A brand name (and) or trade-mark registered by one manufacturer shall not be entitled to registry by another, and the manufacturer having first registered and used the said brand name (and) or trade-mark shall be entitled to it, even should said brand name (and) or trade-mark not be offered for current registration at the time. Nothing in this section shall be construed as debarring the right of any manufacturer to establish his ownership in and prior right of registration of, any brand name (and) or trade-mark, whether said brand name (and) or trade-mark had been previously registered or not.

Sec. 8. No person, company, dealer, or agents shall sell, expose, or offer for sale in this State any pulverized leather, raw, steamed, roasted, or in any other form, either as a fertilizer or fertilizer material, without first making full and explicit statement of the fact in registration with the ——— and furnishing satisfactory proof that the nitrogen is sufficiently available and valuable for the purpose for which sold.

Sec. 9. Be it further enacted, That the ——— shall appoint as many inspectors of fertilizers as in said ——— judgment may be necessary, who shall hold their offices for such time as said ——— in ——— judgment shall think best for carrying out the provisions of this act. The greatest compensation that any one inspector of fertilizers shall receive shall be at the rate of ——— dollars per month and his actual expenses while in the discharge of his duty as such inspector. It shall be their duty to inspect all fertilizers, acid phosphates, chemicals, cotton-seed meal, or other fer-

tilizing material that may be found at any point within the limits of the State, and go to any point when so directed by ———, and shall see that all fertilizers and fertilizer materials are properly tagged.

SEC. 10. Be it further enacted, That each of the inspectors of fertilizers shall be provided with bottles of not less than eight (8) ounces capacity in which to place samples of fertilizers and fertilizer materials drawn by him; and it shall be the duty of each inspector of fertilizers to draw, with such an instrument as shall secure a core from the entire length of the package, such samples of fertilizers and fertilizer materials as he may be directed by ——— to inspect or that he may find uninspected; and in the performance of his duty he shall carefully draw samples as follows: In lots of ten packages or less, from every package; in lots of 10 to 100 packages, from not less than 10 packages; in lots of 100 packages and over, from not less than 10 per cent of the entire number; and, after thoroughly mixing the samples so drawn, he shall, by the method known as "quartering," draw from such thoroughly-mixed sample two subsamples, and with them fill two sample bottles, and shall plainly write on a label on said bottles the number of said sample, and shall also write on the label on one only of said bottles the name of the fertilizer, acid phosphate, or other fertilizer material, also the name of the manufacturers. He shall then seal both of the said bottles, and shall forward to ——— the said samples so drawn by him, stating the number of sacks from which the sample was drawn, and a full report of the inspection written on a form prescribed by ———, which report must be numbered to agree with the number on the bottle; and in said report shall be given the name of the fertilizer or fertilizer material, the name of the manufacturer, the guaranteed analysis, the place where inspected, the date of the inspection, and the name of the inspector; and it shall be the duty of said inspectors to keep a complete record of all inspections made by them on forms prescribed by ———. Before entering upon the discharge of their duties they shall also take and subscribe, before some officer authorized to administer the same, an oath to faithfully discharge all the duties which may be required of them in pursuance of this act.

SEC. 11. Be it further enacted, That a sample of all fertilizers or fertilizer materials, drawn by the official inspectors and filed with the ———, shall be marked by number and delivered by said ——— to the State chemist, who shall make a complete analysis of the same and certify, under same number as marked, said analysis to said ———, which analysis shall be recorded as official and entered opposite the brand of fertilizer or fertilizer material which the mark and number represent; and only the said official analysis of such fertilizer or fertilizer material, under the seal of ———, shall be admissible as evidence in any of the courts of this State on the trial of any issue involving the merits of such fertilizer or fertilizer material.

SEC. 12. Be it further enacted, That the ——— shall have authority to establish such rules and regulations in regard to the inspection, analysis, and sale of fertilizers and fertilizer materials as shall not be inconsistent with the provisions of this act, and as in —— judgment will best carry out the requirements thereof.

SEC. 13. Be it further enacted, That nothing in this act shall be construed to restrict or avoid sales of acid phosphate or any other fertilizer material to each other by importers, manufacturers, or manipulators who mix fertilizer materials for sale, or as preventing the free and unrestricted shipments of material to manufacturers or manipulators who have registered their brands as required by the provisions of this act.

SEC. 14. Be it further enacted, That any person selling or offering for sale any fertilizer or fertilizer material without having first complied with the provisions of this act shall be guilty of a misdemeanor, and on conviction thereof shall be punished as prescribed in ———.

SEC. 15. Be it further enacted, by the authority aforesaid, That all laws and parts of laws in conflict with this act be, and the same are, hereby repealed.

COLLEGE PARK, MD., *November 14, 1903.*

DEAR SIR: In reply to your letter of October 29 I regret to say that I have been so busy with my various duties that I have not had time to give it the attention its importance deserves. The drafting of a State law which will be satisfactory is a difficult matter, but, I think, one to be general in its application would be a much more difficult task, due to the various conditions and different laws already in force in a number of the States. I should think a national law would, of necessity, have to allow considerable latitude in guaranties.

If we could all come to some reasonably close agreement in regard to guaranteed analysis, and that should be embodied in a national law, it would help matters considerably. Since hearing the views of the members of the committee some time ago I am about convinced that this is a hopeless undertaking. I am more fully convinced than ever of the necessity for simplicity in guaranties. Many of our farmers know nothing more than "a ton of phosphate" yet, and have no conception as to any relation between analysis and value. The statement of the phosphoric acid as "soluble" and "available" and "total," and each of these restated in the equivalent in "bone phosphate," and so on with the potash and the nitrogen, is not intelligible to one in a hundred of our farmers, and I doubt if one out of ten of the members of the working force of the agricultural colleges and experiment stations would fully understand it. It is all very well for chemists who have been connected with fertilizer inspection for years to talk about "total nitrogen," "organic nitrogen," "ammoniacal nitrogen," and "nitrate nitrogen," but it is too much for the average farmer—at least in Maryland.

However, I think that national legislation is desirable. The work should be under the Bureau of Chemistry. The repetition of guaranty in different forms and the giving of maximum analyses should, so far as possible, be prohibited. The expense should be defrayed by the Government. The legislation should follow, as nearly as may be, the general trend of inspection already established by the Bureau.

As to just how national legislation should influence State legislation I am not now prepared to state. There are questions of constitutional law, etc., involved, which can best be solved by those who have had considerable experience with them.

Very respectfully,

H. B. McDonnell, *State Chemist.*

St. Louis, Mo., *November 2, 1903.*

My Dear Sir: Your favor of October 29 has been forwarded to me from New York. In reply I would say:

(1) I believe that some legislation of this kind is desirable.

(2) An act which will provide (*a*) the purchaser a minimum guaranty in the plainest terms; (*b*) a revenue great enough to permit of the employment of high-class inspectors and chemists; (*c*) a method of raising this revenue that will be equitable and at the same time tend to prevent unnecessary multiplication of brands; a permanent registration fee (not annual) and a moderate tonnage tax will cover these points; the original registration fee should not be great enough to discourage small manufacturers from registering goods and furnishing local competition; this is especially important in the country west of the Mississippi River, and hence in a law relating to territories; (*d*) that the possessor of the goods as well as the vendor shall be held responsible for obedience to the provisions of the law; (*e*) a model on which local legislation may be based; (*f*) penalties of such kind and extent as to enforce the guaranty and prevent dishonest methods of competition among manufacturers; (*g*) a board of appeal for final decision of differences arising between manufacturers and official inspection bureau; (*h*) flexibility enough to meet new conditions that may arise in the trade.

(3). I believe that there should be no restrictions in regard to the amount of the three essential plant foods in any fertilizer, but the minimum amounts of each should be clearly and definitely stated. National legislation should encourage uniformity in State legislation:

In my opinion the "restriction of commerce" complaint is somewhat overworked. No one questions the fact that some labor and expense are required to conform to the present fertilizer laws. But these laws are the necessary consequences of frauds committed by parties engaged in the fertilizer industry. Fertilizer manufacturers make a fixed charge for "State tax and inspection" and in the cases I have investigated this fixed charge is so much greater than the actual cost that what is an apparent expense is converted into a source of profit. I am aware that provision *d*, holding the possessor, who may also be the consumer, responsible for the obedience to the law, is rather unusual and at present exists in only one State, Indiana. In practice it works admirably; the consumer makes the local agent protect him and the local agent in turn make his principal, the manufacturer, insure him (the local agent) against all costs and penalties arising from failure to comply with the law. In this way a State law is made to reach a manufacturer who is located outside the State. Direct shipments to consumers from outside States are thus reached by a simple provision in the contract, and as the goods are sold on credit, the purchaser is able to deduct from his payments any penalties he has suffered through failure of the manufacturer to fulfill his contract.

While giving due consideration to the somewhat hackneyed complaints about unnecessary restrictions of commerce, we must not lose sight of the causes that gave rise to these restrictions and of the tremendous financial and educational advantages which fertilizer consumers have derived from them. Nor must we lose sight of the fact that an efficient fertilizer law is the best protection that an honest manufacturer can have against unfair competition.

H. A. Huston,
Chemist, German Kali Works.

Auburn, Ala., *November 10, 1903.*

Dear Sir: Your letter of recent date relative to national fertilizer legislation came promptly to hand, and I have carefully noted the various points upon which you desire an expression of opinion by the members of your committee on fertilizer legislation.

With regard to the first point upon which you desire an expression, I would say that national legislation controlling interstate commerce in fertilizers in the District of Columbia and the several Territories would prove quite beneficial, in my opinion, and I also think it highly desirable, sooner or later, to enact legislation which will better facilitate the carrying on of trade in fertilizers between the several States.

From my connection for a number of years with the fertilizer control in this State, I have become aware of the hindrances which have been placed in the way of legitimate traffic and trade in fertilizers by reason of the diverse restrictions and regulations placed upon the traffic in different States. I would say further that as a result of a movement inaugurated by the Association of Commissioners of Agriculture of the Cotton States (including in its membership the official chemists of those States) that practically a uniform law has been adopted in a number of those States, while legislation along the same lines is pending in some of the remaining States. In North Carolina, Tennessee, Georgia, and Alabama (controlling a consumption of more than one and a quarter million tons) a uniform system of branding packages and of stating guaranties is now in force, thereby removing unnecessary and annoying restrictions upon commerce in fertilizers in those States.

With regard to the second question presented for the consideration of the members of the committee, I would say that I am hardly prepared to make definite suggestions as to the exact scope and character of an act which would accomplish the purposes in view.

So far as legislation for the District of Columbia and the Territories is concerned, it seems to me that the essential features of the scheme for fertilizer legislation,[a] proposed by this committee in 1897, could be embodied in the act to advantage.

Among some of the desirable ends to be accomplished by national legislation relating to interstate commerce in fertilizers, may be mentioned the securing of uniform systems of branding and of stating guaranties where shipments are to be made from one State to another; the prevention of the duplication of the same brand or trade name, and the protection of manufacturers having prior claims to the right of use of a given trade name; the prevention of the shipment of condemned or rejected goods from one State to another unless the goods are so branded as to show their true composition or fertilizing value, etc.

National legislation upon these lines would in a short time tend to bring about uniformity in State legislation relative to fertilizer traffic within the individual States, as no State would care to have two different systems for intrastate and interstate commerce.

Yours, very truly,

B. B. Ross,
State Chemist.

The members of the committee seem to be of the opinion that national fertilizer legislation is as much to be desired as is legislation in regard to commerce in foods, for the double purpose of protecting the honest manufacturer and the consumer against the mercenary manufacturer, and especially to unify the provisions of State legislation. The manufacturers and dealers in fertilizers are confronted by exactly the same problem as confronts the manufacturers and distributors of foods. Almost every State in the Union has a food law and a fertilizer law, and no two of these laws were identical, or even harmonious, until recently, when some efforts were made in this direction by several of the States. The result is that the same fertilizers must

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[a] U. S. Dept. Agr., Bureau of Chemistry, Circular No. 3.

be put up and labeled one way in one State and another way in another State. This entails an increased amount of labor on the manufacturer, and the consumer has to pay for it, so the final result is simply to raise the price of fertilizer materials. Your committee is of the opinion that legislation of a national character regulating interstate and foreign commerce in fertilizers and fertilizing materials would be of great benefit in this respect.

I also have the report of the committee on fertilizer legislation of the Association of Agricultural Colleges and Experiment Stations, which is submitted for publication. In the main the two committees are in entire harmony, although the purposes of the two are somewhat different.

REPORT OF THE STANDING COMMITTEE OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS ON UNIFORM FERTILIZER AND FEEDING-STUFF LEGISLATION.

In the course of the past year your committee, as heretofore, has been in correspondence with parties in several States who were interested in the passage of new fertilizer laws or in the amendment of existing ones.

Arizona, Idaho, New Mexico, Nevada, Montana, Wyoming, and Utah have not yet felt the necessity of legislation in this line. In Colorado and Arkansas recent attempts to pass such laws have been defeated. The following reports have been received from some of the other States:

Ex-Director Huston, of Indiana, reports that the existence of the recommendations of this association was of much assistance in connection with steps taken to amend the old fertilizer law in that State. The law as enacted was made to correspond to the recommendations in certain particulars and the other points were practically all left to the discretion of the executive officer, thus rendering it possible to make rules in accordance with the recommendations.

Professor Ladd, of North Dakota, reports that at the last session of the legislature in that State a fertilizer law was enacted, and that the bill was drawn in accordance with the recommendations of this association, which he says were very helpful in the preparation of the bill "and in securing the necessary legislative action thereon."

R. E. Rose, State chemist, Tallahassee, Fla., writes that the law in that State has recently been amended to conform, in so far as possible, with the recommendations concerning uniformity. He adds that the recommendations were of material service.

Prof. F. B. Mumford, of Missouri, reports that the law in that State has been amended recently and that the recommendations were of "much assistance."

President McBryde, of Virginia, reported July 4, 1903, the changes in the law in that State were then being considered and that amendments in the line of the recommendations were being urged. In conclusion he says: "It follows, therefore, that your recommendations will be helpful in securing the legislation needed."

Director Armsby reports that the recently amended law of Pennsylvania conforms very largely in substance to the recommendations.

Director Soule, of Tennessee, states that a new law was passed in that State in April, 1903. The law was drawn with the object of making it conform with the recommendations of the associations, but a few amendments were made not in harmony therewith which it is believed weakened the law. He adds that it is hoped later to secure such amendments as will make the law conform to the original draft, and that "Had it not been for the existence of the recommendations, it would probably not have been possible to secure the passage of the present law."

Director J. F. Duggar, of Alabama, writes under date of July 7 that in that State "the old law has been replaced this year by a new one, which embodies the recommendations of the Association of American Agricultural Colleges and Experiment Stations and of the Association of Official Agricultural Chemists," and that "the recommendations alluded to have had much weight in securing the revision of legislation along this line."

After careful consideration of the subject, your committee submits the following recommendations regarding laws regulating the sale of feeding stuffs.

(1) That for the purpose of defraying the expenses of feeding-stuff inspection the State should make a direct appropriation, or where this is impracticable a brand tax should be levied. In view of the experience of Maine and Vermont a tonnage tax is not to be recommended.

(2) That the following materials should be exempt from the provisions of feeding-stuff laws; hays and straws and whole unmixed seeds, such as wheat, rye, barley,

oats, Indian corn, buckwheat, broom corn, peas, and the unmixed meals of the entire grains of such seeds.

(3) The term "concentrated feeding stuff" should include linseed meals, cotton-seed meals, cotton-seed feeds, pea meals, cocoanut meals, gluten meals, gluten feeds, maize feeds, starch feeds, sugar feeds, dried brewers' grains, dried distillers' grains, malt sprouts, hominy feeds, cerealine feeds, germ feeds, rice meals, oat feeds, corn and oat chops, corn and oat feeds, corn bran, ground beef or fish scraps, condimental foods, poultry foods, stock foods, patented proprietary or trade-mark stock and poultry foods, and all other materials of a similar nature not included in section 2 above. Where practicable the by-products from the milling of wheat, rye, and buckwheat should be included under the requirements of the laws.

(4) That a legible printed statement should be affixed to or printed on each package containing a feeding stuff named in section 3, giving the net weight of the package, the name and address of the manufacturer or importer, the name, brand, or trade-mark under which the article is sold, and the guaranteed analysis showing the percentage of crude protein and of crude fat and a maximum of fiber which shall not be exceeded.

The law should provide that the chemical analysis, including the determinations of crude fiber, crude protein, and crude fat shall be made by the official methods of the Association of Official Agricultural Chemists.

If the feeding stuff is sold in bulk or put up in packages belonging to the purchaser, the agent or dealer shall furnish him with a certified statement of the net weight of the lot, the name and address of the manufacturer or importer, the brand or trade-mark under which said article was sold, and the percentage of crude protein and crude fat which said article is guaranteed to contain, as determined by the official methods of the Association of Official Agricultural Chemists.

(5) That a certified copy of the statement in section 4 above be filed with the executive officer each year.

(6) That the law should contain a penalty by fines only for violations of its provisions.

The committee recommends to the Association of American Agricultural Colleges and Experiment Stations the adoption of the recommendations 1 to 6, inclusive, with the suggestion that this or some other committee should be instructed to use its efforts to secure the end in view by using its influence to aid in securing uniform legislation in the several States.

Respectfully submitted.

H. J. Wheeler.
Chas. D. Woods.
E. H. Jenkins.
H. P. Armsby.
M. A. Scovell.

I would like also to call attention to the suggestion which was made to me by Mr. W. S. Myers, director of the nitrate of soda propaganda in this country. Under date of November 12, he writes as follows:

Dear Sir: It has occurred to me, as doubtless it has to you, that the practice of our experiment stations in reporting available phosphoric acid and potash, and failing to report available nitrogen, does not disclose to the consumer the value of the fertilizer from either a commercial or a merely agricultural standpoint. The official methods now in use are adequate at least to make a preliminary classification of the nitrogenous content of fertilizers, but the usual practice of the station laboratories is to determine and report total nitrogen only. Is this not most unfortunate for the farmer, for whether the phosphoric acid is worth 2 cents or 4 cents a pound does not seem as of great moment as whether the nitrogen is worth 4 cents or 16 cents a pound? Even though the nitrogenous content is small in the average commercial fertilizer, small percentages at high prices cut a large figure in valuations.

The purpose of this note is to ask the Association of Official Agricultural Chemists to consider recommending by resolution the practice of putting into use the methods already adopted for nitrogenous fertilizers. At the present time most of the stations are not willing to take the trouble. I am aware that methods are under investigation for the classification of organic nitrogen. Does not this seem rather premature in view of the failure to put into practice the simple methods already adopted? To put the case concretely, from my standpoint, the determination of nitrates of ammonia salts and of organic nitrogen should be made in each fertilizer whenever found.

Very sincerely, yours,

William S. Myers.

It appears from the views expressed by the members of the committee and others that the general opinion is that some national legislation should be enacted, but there is no definite expression as yet by the committee as to the form that this legislation should take. I therefore move that the committee on fertilizer legislation be instructed to confer with manufacturers and distributers and with such other persons as may be interested in the matter and report at the next meeting the form that national legislation should take with a view to preparing a tentative bill and urging its enactment into a law.

The motion was adopted.

Mr. Wiley. I would like to offer two resolutions. The first one pertains to the advisibility of associating the National Bureau of Standards with the work of this association. I have had a conference with the chief chemist of the Bureau of Standards, and, while he would not commit himself on account of his inability at that time to consult with the director of the Bureau, he looked very favorably upon the proposition which I made, to the effect that, as the Bureau of Standards was now established by law as the final court of resort for all matters relating to standards, it would be desirable for us to collaborate on standard methods of analysis.

It can do no harm, therefore, to express our desire for such collaboration if it can be secured after consultation with the officials of that Bureau. I therefore move the following resolution:

Resolved, That the Bureau of Standards be invited to cooperate with the referees of the association in so far as possible in establishing the accuracy of standard methods of analysis.

Adopted.

[The following letter has been received from Director Stratton, and the referees are therefore requested to ask the Bureau of Standards to collaborate in their work. H. W. W.]

December 8, 1903.

Dear Sir: I beg to acknowledge the receipt of your letter of December 4, transmitting resolutions passed by the Association of Official Agricultural Chemists inviting the Bureau of Standards to cooperate with the association in establishing the accuracy of standard methods of analysis. In reply I would state that the Bureau of Standards will be pleased to cooperate with the association in every way consistent with its functions. This question is one in which the Bureau, and especially our chemical section, is very much interested.

Respectfully, S. W. Stratton, *Director*.

Mr. Wiley. The second resolution which I propose to offer relates to a very important part of the work of agricultural chemistry recently taken up by the Department of Agriculture, which has realized the importance to agriculture of the cultivation of medicinal plants. Agriculture, of course, includes forestry and horticulture, and the territory of the United States is now so extended that within the jurisdiction of the United States almost every plant which is used in medicine is grown. Hence the action of Congress in establishing two different forms of investigation of medicinal plants in the Department of Agri-

culture—one relating particularly to the botanical side of the work in the discovery and introduction of new and useful medicinal plants within the jurisdiction of the United States, and the other in establishing a drug laboratory where such plants can be analyzed and their medicinal properties determined. Inasmuch, therefore, as that work has been recognized by Congress as a part of the official work of the Department of Agriculture I move the following resolution:

Resolved, That a referee on the analysis of medicinal plants and drugs made therefrom be appointed by this association.

Adopted.

Mr. Van Slyke. If there is no objection, I would like to offer two resolutions. The first one relates to a request to be made of the secretary in regard to printing the recommendations that are passed by the association. According to our constitution a recommendation which carries with it a change of method can not be acted upon finally by the association until the members have had an opportunity to test the method as modified. That, as matters now stand, practically involves a postponement of a year, or, practically, two years. If we can place in the hands of the members within a comparatively short length of time after the adjournment of each meeting the results of the association's work in the form of recommendations, there will be an opportunity to test these methods, and the time of adoption will be hastened. I therefore move the following resolution:

Resolved, That the secretary be requested to print the changes in methods and instructions recommended by the association and distribute them to the members at as early a date as is practicable.

Adopted.

Mr. Van Slyke. The other resolution I have in mind relates to the place of meeting for next year. The constitution provides the association shall determine the place of meeting. I move that the executive committee be authorized to select a place of meeting next year and that they be requested to consider the feasibility of having that meeting in St. Louis.

The executive committee already has the power to determine the time. The association, unless it delegates the power to the committee, determines the place.

Mr. Huston. As a resident of the city of St. Louis I feel sure you would receive a very cordial welcome if you should meet in that city. There will, of course, be many congresses there next year, and our meetings, if arranged to accord with some others, would, I think, be doubly interesting. The Association of American Fertilizer Manufacturers have arranged to meet there, and I am sure the meeting will be unusually interesting to the members of this association, as it will be of a scientific nature.

Mr. WILEY. We have a good precedent for this action in the two meetings that have been held outside of Washington. All who were present remember with pleasure the Chicago meeting in 1903, and the San Francisco meeting in 1899, which was a far greater success in point of attendance than anyone had anticipated. I think we are rather tardy in honoring the man who did so much to make that meeting a success—our president-elect, Mr. Jaffa. I am heartily in favor of the suggestion made, and am sure the executive committee will be glad to consider the suggestion to arrange for the next meeting in St. Louis.

The motion of Mr. Van Slyke was carried.

Mr. COATES. Have any steps been taken to secure the revision of Bulletin No. 46?

Mr. WILEY. We have ready for mortising or resetting many of the changes that have been made since Bulletin No. 46 was printed. By reason of the expense of resetting, it is deemed wise to wait until the methods had settled into more definite form. You all know how rapidly the methods were changed and how slowly, fortunately, they are now changed. We now hope to issue an entirely new edition of Bulletin No. 46 very soon.

The PRESIDENT. Is it the intention to embody in the revised edition the methods as published in Bulletin No. 65 for the analysis of foods and food products?

Mr. WILEY. We might do that. Some of these methods, however, are only offered for trial and could hardly be made official or even provisional.

Mr. BIGELOW. It might be well to have an expression of opinion on this point by the association, as to whether it would be desirable to print all the methods together. We have now several classes of chemists in the association; many of the experiment station chemists have no interest in the food methods, except perhaps to have them accessible, and others, belonging to the boards of health and dairy and food commissions, have no interest in the other methods, such as those on soils and fertilizers. If the two lines of work are published together it would make a bulletin of about 250 pages. At the same time there are some who are interested in both lines of work. Another point is that the food methods will change radically and in detail for some time to come. It is probable that they will not be in a shape to be adopted by the association finally for several years.

The PRESIDENT. Considering the remarks of Mr. Bigelow it seems advisable in the case of the present revision, at least, to print the food methods separately. I would like to have an expression of opinion from other members of the association.

Mr. MORSE. I should prefer to have them separate. It is more

convenient on account of the size, and as changes are sure to be made in the food methods it would take longer to get these into print if they are all printed together.

Mr. McDonnell. It seems to me it should be left largely in the hands of the Secretary. From what has been said it appears, however, to be more convenient and better in every way to have two bulletins.

Mr. Williams. What action is going to be taken, if any, with reference to printing the Dancy and Battle conversion tables?

Mr. Wiley. If these tables are offered as a part of the proceedings, they will be published as such. I think they were offered by the authors, without any interest in the copyright, for the use of the association.

Mr. Williams. They only asked credit for their work.

Mr. McDonnell. I have no objection to printing these tables, but I think those printed by the Maryland station, which I have submitted, are more compact and convenient, and it might be well for the association to compare the two before printing.

Mr. Wiley. As the constants of nature are constantly changing it might be well, if the association does ask for the publication of tables which have been calculated for a number of years, to recognize the fact that a number of new elements have been discovered during that time and some of the old ones have been reduced in dignity and some increased, and to have a careful revision of the calculations before printing them.

The President. I would suggest that a committee be appointed, authorized to prepare either from the Dancy and Battle tables or Mr. McDonnell's bulletin tables for publication in the Proceedings.

Mr. Hardin. Down South the chemists still use the Dancy tables for nitrogen and phosphoric acid. The potash tables we have not been able to use for several years, as a new factor has been adopted. Most of us prefer the form of these tables, and I think myself that they are the best that have ever been published. But in getting them up again they would have to be revised.

Mr. Huston. Perhaps it would not be well to print any of these right away. There is an excellent set of tables in use in the Indiana laboratory, calculated by Mr. Jones, which brings the potash up to the present weight, and the table for nitrogen is exceedingly useful, as it is based on the official methods in use. A committee to decide what tables should be published would be very useful. I am quite sure that the Indiana tables could be used, either in whole or in part, if acceptable to the committee.

Mr. Wiley. I move that the president be requested to appoint a committee of three, of which Mr. H. B. McDonnell shall be the chairman, to consider the matter of publishing in the Proceedings of the

association calculation tables, and that the committee be authorized to submit its report to the secretary of the association with recommendation.[a]

Adopted.

Mr. Wiley. I would like to call the attention of the association to the fact that this is a record-making meeting in the point of attendance. Over 140 members have registered during the convention. This shows what a vigorous growth the association is now having. I would like to say a word as one of the Nestors of the association, with a record which no one else has, of having been present at every meeting since its organization. What we need now particularly in this association is the more vigorous cultivation of skill in analysis. Altogether too few of our members take part in the referees' work.

There are two benefits which accrue to every member of the association taking part in the work of the referees; one is the benefit he confers upon the association, and the other the benefit he will confer upon himself, because if he be in any way inaccurate, or if he fail to reach the standard of excellence of this association, he will be certain to find it out the moment he undertakes collaborative work and be able to correct any fault he may have.

In the establishment of standards and the securing of data for outlining methods accuracy is absolutely indispensable. The formation of theories is not a difficult thing if you have the right basis; and I recall especially the remarks made by Professor Crookes, in his address before the Berlin congress last June, that no theory of any kind is of any value unless every fact which is to be correlated in that theory finds a place there. And so it is that in the great discussions of the problems relating to agricultural chemistry we must be sure first of all of the ground on which we stand.

As I look over this association I realize, what we see everywhere, that it is the young men who rule the world. We old chaps are altogether in the minority. Presumably it is the young men who need the training most. While they are just out of college and have done a certain amount of practical work, they have not had the necessary training for such investigations, and I especially appeal to the young men—and to the young women too, because we are seeing evidences of the other sex breaking over into our ranks—I earnestly appeal to them to take an active part in the work of the referees. I would not have a chemist in my Bureau who would not take part in this referee work. I not only require it but I give every opportunity for doing such work. I would say to the trustees of every station, "Send your chemists to the meetings." As a matter of fact we do have the greater part of these stations represented, but they ought all to be represented, and the station chemists should be afforded every facility to

[a] The report of this committee is given on page 220.

engage in collaborative work. The Secretary of Agriculture affords every facility to the chemists in the Department of Agriculture and encourages them to engage in the work of this association. Long before it was made by Congress his advisor he did that, and of course he is still more interested now. We are the only association of this kind that has any official standing by act of Congress, and therefore we are under special obligations to the people of this country to perfect ourselves in everything that lies at the foundation of our science. So, let us have more collaboration, especially among the younger members of the association, and instead of two or three people reporting results, let us have thirteen or fourteen, even if four-fifths of them have to be sent back as not fit for publication. [Applause.]

The PRESIDENT. I appreciate very much what Doctor Wiley has said, and he has emphasized certain remarks that I made in the opening address. It is very regrettable that our referees have so little cooperation in their work. Every individual of this association ought to make it his business to do some work on one of the subjects under consideration, and I think the time is coming when those who do not will be considered as deadwood in the association.

I also wish to call special attention to another point which I think has been mentioned in regard to the sugar work. This is the graduation of the volumetric apparatus at too low a temperature, 15.5°. I think something should be done toward having the graduation made at some temperature more suitable to that of our laboratories.

Is the committee on resolutions ready to report?

Mr. MCDONNELL. In the absence of the chairman of this committee, I move that the secretary extend to the officers of Columbian University, the Cosmos Club, and to the Secretary of Agriculture our thanks for the courtesies extended to the association.

Adopted.

Mr. BIGELOW. Mr. Walter Bernays has asked me to call the attention of the association to the fact that no effort has been made as yet to establish methods for the examination for disinfectants, and it is a subject with which the boards of health are having much trouble, and they would like to have it taken up. It is a question whether this subject would come within the province of the association, and some expression of opinion is desired as to whether it would be desirable to consider it.

Mr. COATES. A few years ago there was a yellow fever scare in a town in Louisiana, and the place was flooded with bleaching powder. I analyzed some of it and found it contained less than 2 per cent of available chlorin, and could not find any specimen in town which contained more than that. This question is going to be more and more

important as time goes on, and I would like very much to see Mr. Bigelow's suggestion acted upon.

Mr. Bigelow. I move that a referee be appointed to take up this question.

Mr. Haywood. As we have a number of reporters now and have just created a new one on drugs, it seems hardly necessary to appoint a referee for this special work. The work would be very much along the line of our present investigations on insecticides. I think it would be better to refer this subject to the referee on insecticides, and therefore offer an amendment to the effect that the establishment of methods for the analysis of disinfectants be referred to the referee on insecticides and fungicides.

Mr. Bigelow. I accept the amendment.

The motion as amended was passed.

The President. Is the chairman of Committee A on recommendations, ready to report?

REPORT OF COMMITTEE A ON RECOMMENDATIONS.

B. L. Hartwell, *Chairman.*

(Phosphoric acid, potash, nitrogen, soils, ash, and insecticides.)

1. Phosphoric Acid.

No recommendations were made by the referee, and those made by the associate are practically covered by the recommendations on soils.

2. Potash.

It is recommended—

1. That the question of the further trial of the modification requiring a slightly acid solution and neutralization with sodium hydroxid be left to the discretion of the referee. (Adopted.)

2. That the association take some action to ascertain whether the members desire to determine moisture in potash salts by drying at a stated temperature or by ignition, and that the study of the method thought most desirable be continued with a view to obtaining more concordant results for water in potash salts.

(The committee, without action, presented paragraph 2 to the association, which referred the whole question to the referee for 1904.)

On motion by Mr. Ross, the association adjourned at 12.50 until 2 o'clock, the report of Committee A to be completed at the afternoon session.

SATURDAY—AFTERNOON SESSION.

The President. On the committee to report to the secretary on calculation tables, of which Mr. McDonnell is the chairman, I will appoint Mr. Williams and Mr. Ross.

REPORT OF THE COMMITTEE ON CONVERSION TABLES.

[The following letter was submitted to the secretary of the association, in accordance with the resolution passed, together with the tables selected. It was not clear,

however, from the instructions given, whether the association desired the secretary to take action on the report of the committee and publish the tables recommended or merely to receive the report; and further, there seemed to be a difference of opinion as to whether the tables were to be printed in the revised edition of Bulletin 46, on Methods of Analysis, or in the Proceedings. In view of these facts the secretary submits the report herewith and holds the tables recommended for the action of the association at the meeting of 1904. H. W. W.]

COLLEGE PARK, MD., *February 17, 1904.*

DEAR SIR: On behalf of the committee appointed by the Association of Official Agricultural Chemists, consisting of Mr. B. B. Ross, Mr. C. B. Williams, and myself, to select conversion tables for publication in the Methods of Analysis, I beg to report that a majority of the committee have decided to recommend McDonnell's tables, but think that in view of the fact that the atomic weights have been revised since the tables were first published, making slight changes in the factors, they should be recalculated and a protein table added. This I have done with the assistance of Messrs. R. H. Kerr and William R. Wharton. The revised tables are sent herewith.

Very respectfully,

H. B. MCDONNELL,
Chairman Committee on Conversion Tables.

Mr. WINTON. Referring to Mr. Hillebrand's report of this morning I wish to move the following resolution:

Resolved, That the Chair be instructed to appoint a committee from this association to cooperate with the committee of the American Chemical Society in the matter of testing chemicals for purity.

Adopted.

The PRESIDENT. I will appoint Mr. Kebler, Mr. Winton, and Mr. Kilgore on this committee.

The report on tannin will now be received.

REPORT ON TANNIN.

By G. A. KERR, *Referee.*

Pursuant to the recommendations made in 1902, the referee sent to 16 chemists samples of fluid chestnut and solid quebracho extracts for the purpose of—

(1) Confirming the use of a single filter-paper in determining soluble solids.

(2) Investigating the Proctor correction for filter paper absorption, as adopted by the I. A. L. T. C.

(3) Ascertaining the effect, if any, of using a specified dilution in comparison with the variation now allowed by the official method.

Samples of acidified hemlock liquor were also sent out, for the purpose of further testing the present provisional method, and also an experimental method, suggested by W. H. Teas, for the determination of acids in tanning liquors.

An effort was made by the referee to procure a dry chromed hide powder for comparison with the present wet hide powder method, but this effort was unsuccessful owing to the impossibility of procuring in time for this year's work, a hide powder which could be dried with the retention of its absorptive qualities.

STUDY I.

Instructions were given to determine the total solids, soluble solids, and insolubles in samples No. 1 and No. 2, by the association method.[a] The results returned are contained in Table I.

[a] U. S. Dept. Agr., Bureau of Chemistry, Circular No. 8, revised.

TABLE I.—*Determinations of total and soluble solids and insolubles by the provisional method.*

No. of determination.	Chestnut extract (A).			Quebracho extract (B).		
	Total solids.	Soluble solids.	Insolubles.	Total solids.	Soluble solids.	Insolubles.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	37.16	36.18	0.98	86.61	79.56	7.05
2	37.43	36.40	1.03	87.61	81.55	6.06
3	37.62	36.92	.70	88.49	82.00	6.49
4	37.01	36.15	.86	86.99	81.80	5.19
5	37.78	36.69	1.09	87.50	82.30	5.28
6	37.42	36.35	1.07	86.76	80.48	6.28
7	37.20	36.14	1.06	86.55	79.82	6.73
8	37.73	36.95	.78	88.72	81.12	7.60
9	37.69	36.43	1.26	88.55	81.57	6.98
10	36.37	35.16	1.21	86.35	80.28	6.07
11	38.37	36.88	1.49	85.09	78.91	6.18
Average	37.43	36.38	1.04	87.20	80.85	6.35
Maximum	38.37	36.95	1.49	88.72	82.30	7.60
Minimum	36.37	35.16	.70	85.09	78.91	5.19
Greatest difference	2.00	1.79	.79	3.63	3.39	2.41
Greatest variation from average	1.06	1.22	.44	2.11	1.94	1.24

In the soluble solids column, chestnut extract (A), the greatest difference from the average quantity of soluble solids found by 11 analysts is 3.36 per cent of the whole. In the quebracho extract (B) it is 2.39 per cent. While these figures confirm last year's work with reference to the adoption of the single-filter paper for filtration of soluble solids, they do not convey an adequate idea of the variation between the highest and lowest percentage of soluble solids found by the different analysts.

Under A the greatest variation is 1.79 per cent, and under B, 3.39 per cent. When it is considered that these differences have a direct bearing upon the tannin figure, the weakness of the present method of determining soluble solids is very apparent.

An examination of the columns giving total solids and insolubles reveals a condition equally unsatisfactory, and makes the seat of trouble somewhat uncertain, commencing as it does with a variation of over 5 per cent and 4 per cent in the total solids columns, and ending with one of 112 per cent and 37 per cent in the insoluble columns, the indication would seem to be that the entire method, as applied to these items, needs revision.

From personal observation and the comments of the collaborators in this work the referee is of the opinion, however, that the method is not entirely accountable for the differences in results, as it has been demonstrated that with close attention to detail of manipulation and operation much more concordant figures than the above can be obtained. Attention is especially called to the necessity for protecting fluid extracts against loss by evaporation while weighing same for dilution; this also applies to the absorption of moisture by pulverized dry extracts.

The drying of residues, and the loss from evaporation that ensues during the filtration of soluble solids, also require closer attention. The error from the latter cause is much greater, apparently, than has been supposed, and the referee would recommend that as a specific means of guarding against it the following be embodied in Paragraph IV, Soluble solids,[a] of the method: "To guard against loss by evaporation, flasks receiving filtered liquor shall be stoppered with a perforated rubber stopper, and the funnel stem inserted through perforation. Funnels shall be covered

[a] U. S. Dept. Agr., Bureau of Chemistry, Circular No. 8, revised.

during filtration with a glass plate, having an orifice only sufficiently large to admit air required by displacement of liquor."

It is further recommended that the last sentence of Paragraph II, Quantity of material, [a] be changed to read: "In the case of extract weigh in weighing bottle fitted with ground-glass stopper, such quantity, etc."

Mr. Veitch makes the following comment:

I have never been satisfied that the change from the double filter to a single one, was a wise one, owing to the fact that kaolin is so apt to run through a single paper. It has usually been my experience, even after rejecting the prescribed volume of liquor, that some kaolin still passes through, and may be best seen by carefully tilting the flask in which the filtrate is received.

Study II.

This experiment consists of a trial of the filter paper absorption correction as suggested by Mr. Proctor and adopted by the I. A. L. T. C. The following instructions are copied verbatim from the official method of the above association, except that the method of filtration was carried out according to the method of this association.

(*a*) To determine the correction, filter clear about 500 cc of the tannin solution, of the strength prescribed for analysis. After thoroughly mixing, 50 cc is to be evaporated, to determine soluble solids (1). A portion of the remainder is then to be filtered in precisely the same manner as for (1), and 50 cc of the filtrate is to be evaporated for soluble solids (2).

(*b*) Deduct (2) from (1) and the difference found is the correction which must be added to the soluble solids (1) to give the corrected soluble solids.

(*c*) In proceeding with these filtrations use 2 grams (weighed) of kaolin, which is first to be washed with 75 cc of the liquor by decantation and then washed onto the filter with a further quantity of liquor; reject the first 150 cc which passes through in each filtration. Keep the filter full and guard against evaporation.

The results of this study are given in Table II.

Table II.—*Determination of correction for soluble solids.*

Determinations.	Chestnut extract.					Quebracho extract.				
	Total solids.	Soluble solids (1).	Soluble solids (2).	Difference.	Corrected soluble solids.	Total solids.	Soluble solids (1).	Soluble solids (2).	Difference.	Corrected soluble solids.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1	37.35	36.35	36.19	0.16	36.51	86.92	79.67	79.23	0.44	80.11
2	37.43	36.45	36.14	.32	36.77	87.61	81.66	80.49	1.17	82.83
3	37.63	37.33	37.06	.27	37.60	88.52	84.25	83.48	.77	85.02
4	36.37	35.32	34.94	.38	35.70	86.35	80.34	79.89	.45	80.79
5	36.95	36.15	35.63	.52	36.67	87.05	81.85	80.49	1.36	83.21
6	37.78	36.69	36.44	.25	36.94	87.58	82.30	81.95	.35	82.65
7	37.42	36.63	36.52	.11	36.74	.....	81.30	80.55	.75	82.05
8	37.37	36.73	36.48	.25	36.98	86.74	81.01	80.14	.87	81.88
9	37.45	36.73	36.70	.03	36.76	88.39	81.72	80.50	1.22	82.94
10	37.64	36.85	36.48	.37	37.22	88.32	83.40	82.51	.89	84.29
11	.....	37.17	36.82	.35	37.52	.....	80.30	80.15	.15	80.45
12	.....	37.06	36.02	1.04	38.10	.....	77.13	76.93	.20	77.33
Average	37.33	36.62	36.28	.33	36.95	87.497	81.24	80.52	.718	81.96
Maximum	37.78	37.33	37.06	1.04	38.10	88.52	84.25	83.48	1.36	85.02
Minimum	36.37	35.32	34.94	.03	35.70	86.35	77.13	76.93	.15	77.33
Greatest difference	1.41	2.01	2.12	1.01	2.40	2.17	7.12	6.55	1.21	7.69
Greatest variation from average	.969	1.30	1.34	.70	1.259	1.47	4.11	3.59	.64	4.63

The foregoing results indicate the necessity of having ultimately a correction unless a nonabsorbent filter paper is secured or the filter paper now in use can be thoroughly tanned by the liquor passing through before the portion necessary for evaporation is taken. At present, however, it is plain, especially in the case of quebracho extract in Table II, that the method employed to ascertain this correction augments to an exaggerated extent the error already existing in the determination of soluble solids, which has the same bearing on the tannin content as the correction is intended to have. In consideration of this condition the referee would suggest that until more uniform results are obtained on the items under discussion further experiments in this line be deferred.

In both cases the differences cover a wider range than in Table I, but with the quebracho extract in Table II the differences are abnormal. It seems to be the consensus of opinion that this difference is largely due to loss from evaporation during the extended length of time required to filter the necessary quantity of liquor for the determination. In this connection it is interesting to note that the precautions employed by the various analysts to guard against evaporation do not seem to have been very effective, which fact emphasizes the necessity of adopting specific means for its prevention, as suggested by the referee in discussing the first experiment. The analysts make the following comments:

Mr. Veitch.—From the results of studies 1 and 2 it appears that the absorptive power of the filter is not satisfied by filtering 150 cc of solution. This is particularly apparent in the case of quebracho.

Mr. Mosbaugh.—Just after Mr. Proctor published the method I gave it some attention, and obtained satisfactory results in all extracts with the exception of solid quebracho. Nevertheless I am in favor of its being adopted, as it is well known that filter paper absorbs more or less tannin and this method of correction apparently remedies the evil.

Mr. Reed.—Personally I am not in favor of the method.

Mr. Teas.—I think the evaporation, taking place during filtration, accounts for the high soluble solids.

Study III.

For the third study the following instructions were given; results will be found in Table III:

Using official samples Nos. 1 and 2 determine total solids, soluble solids, and insolubles in each according to the provisional method, except as to dilution.

(a) For dilution of sample No. 1 use exactly 1.6 grams per 100 cc.

(b) For dilution of sample No. 2 use exactly 0.6 grams per 100 cc.

Weigh out the portion of sample No. 1 for dilution, in a covered vessel, to guard against evaporation. It is preferable to weigh out approximately the quantity required and dilute so that each 100 cc will contain 1.6 grams of the extract.

It having been suggested to the referee that the range of variation in the quantity of tannin admissible in dilutions according to the association method was too great to give uniform results (there being a difference of over 28 per cent between maximum and minimum), this experiment was undertaken to determine, by comparison with Table I, the value of results obtained by using a specific quantity of total solids. Comparing these tables, we find the results to be in favor of the specified quantity test; slightly so in sample 1, Table III, but more markedly so in sample 2.

The improvement will, perhaps, scarcely justify any change in the official method for the present, but the referee would suggest that the experiment be repeated with varying quantities of extract until it is ascertained whether or not the total solids contained by an extract form a better basis for dilution than the assumed tannin content. As there is evidently some error in drying residues, caused by the varying quantity of total solids in different dilutions, it seems to the writer that it would be easier to adjust the quantity of residue to be dried to meet the present conditions

governing drying than to alter the method of drying to suit the difference in quantity of residue obtained by the association method now in use, inasmuch as this item has been more thoroughly worked out than any other in tannin analysis, excepting non-tannins.

TABLE III.—*Determinations by association method with modifications as to dilution.*

Determinations.	Sample 1 (chestnut extract, 16 grams per liter).			Sample 2 (quebracho extract, 6 grams per liter).		
	Total solids.	Soluble solids.	Insolubles.	Total solids.	Soluble solids.	Insolubles.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	37.31	36.26	1.05	87.31	81.28	6.03
2	36.95	36.16	.79	86.75	79.61	7.14
3	36.22	35.23	.99	88.63	80.76	5.54
4	37.74	37.06	.68	88.35	81.41	6.94
5	37.73	36.70	1.03	87.60	81.87	5.73
6	36.89	36.09	.80	86.50	80.51	5.99
7	37.25	36.00	1.25	86.83	80.66	6.17
8	37.30	36.27	1.03	86.80	79.87	6.93
9	37.63	36.49	1.14	87.58	80.56	7.02
10	37.65	36.87	.78	88.83	82.07	6.76
11	37.65	36.60	1.05	88.88	81.37	7.51
12	37.97	36.68	1.31	.....	.....	.....
Average	37.36	36.36	.99	87.43	80.90	6.52
Maximum	37.97	37.06	1.31	88.88	82.07	7.51
Minimum	36.22	35.23	.68	86.30	79.61	5.44
Greatest difference	1.75	1.83	.63	2.58	2.46	2.07
Greatest variation from average	1.14	1.13	.31	1.45	1.29	1.08

STUDY IV.—ACIDITY OF LIQUORS.

For these experiments a sterilized neutral hemlock liquor (specific gravity 1.015), to which 0.15 per cent of acetic acid had been added, was sent out, and analysis requested by the following methods:

(*a*) By provisional method, paragraph 8, circular No. 8 revised, Bureau of Chemistry.

(*b*) To 100 cc of diluted liquor (prepared as in the provisional method) add 2.5 grams chemical pure animal charcoal, stir every fifteen minutes for two hours, filter, and titrate an aliquot portion with N/10 alkali. For results see Table IV.

TABLE IV.—*Total acidity of liquors as acetic acid.*

Determinations.	Provisional method.	Experimental method.
	Per cent.	*Per cent.*
1	0.195	0.161
2	.178	.137
3	.135	.150
4	.150	.156
5	.183	.149
6	.174	.140
7	.168	.144
8	.156	.120
9	.165	.105

TABLE IV.—*Total acidity of liquors as acetic acid*—Continued.

Determinations.	Provisional method.	Experimental method.
	Per cent.	*Per cent.*
10	0.170	0.120
11	.180	.120
12	.148	.140
Average	.1668	.1368
Maximum	.195	.161
Minimum	.135	.105
Greatest difference	.060	.056
Greatest variation from average	.0318	.0318

It will be noted that the provisional method gives results about 11 per cent higher than theory, and the experimental method the same amount lower.

By referring to Table IV in the referee's report for 1902[a] it will be seen that practically the same results were obtained. The percentage of variation from the average is very similar, although the liquor for 1902 evidently contained about twice as much acid as that sent out this year.

In the opinion of quite a number of analysts this provisional method of determining the acidity of liquors is very satisfactory in tannery practice, for which it is almost exclusively used, especially as to the concordance of the results obtained. Notwithstanding this the referee would suggest that further experiments be conducted with reference to the loss of acid presumably held or neutralized by the charcoal in method "b."

COMMENTS OF ANALYSTS.

Mr. Mosbaugh.—The acid determination I find does not cover the range of liquors found in sole-leather yards. While the results obtained are concordant and uniform, the method is not applicable to liquors higher in specific gravity than 1.030. As it is sometimes necessary to determine the acidity of the new as well as of the sapped liquors, it is impossible to get a filtrate sufficiently clear for titration, unless a weaker dilution is made or more charcoal used, and in either case the results are not as satisfactory as when the same amount of charcoal is used. I find that by carefully using gelatine in connection with charcoal very good results are obtained and approximately the correction as used for charcoal method holds out.

Mr. Stilwell.—In titrating liquor in Study IV I used for the alkali sodium hydrate, checking with limewater. I do not get good results when using N/10 soda, and of the three alkalis prefer limewater.

Good results are obtained by using 2 to 2.5 grams of charcoal and running in a shaker for from to two to three minutes. Decolorization is satisfactory. The best concentration is 0.2 to 0.25 grams of tannin per 100 cc of tan liquor.

In addition to the determinations prescribed in Study IV, Mr. Reed also determined the acidity with charcoal furnished by last year's referee (Mr. Teas), with the following results:

(*a*) Provisional method: 2.75 cc N/10 sodium hydrate to neutralise 40 cc of solution. Total acidity = 0.206 per cent as acetic acid.

(*b*) Cold charcoal method; 2.25 cc N/10 sodium hydrate to neutralise 40 cc of solution. Total acidity = 0.169 per cent as acetic acid.

Method (*a*) using 2.5 grams of charcoal furnished by this year's referee, instead of two grams as provided, gave a clearer filtrate in the former than in the latter case;

[a] U. S. Dept. Agr., Bureau of Chemistry Bul. No. 73, p. 81.

2.3 cc N/10 sodium hydrate to neutralise 40 cc of solution. Total acidity = 0.173 per cent as acetic acid.

Method (*a*): 2 grams of charcoal = 0.195 per cent total acidity.

Method (*a*): 2.5 grams of charcoal = 0.173 per cent total acidity.

Loss due to 0.5 gram of charcoal = 0.22 per cent total acidity.

Loss due to 2.0 grams of charcoal = 0.88 per cent total acidity.

Corrected total acidity = 0.195 per cent + 0.088 per cent = 0.283 per cent.

RECOMMENDATIONS.

The only recommendations are those given under Study I, page 222. Suggestions as to future work are also made under the several studies.

Mr. STILWELL. I would like to suggest that 9 hours' extraction be made official. In ordinary tannery practice it does not seem advisable to extend the time very much over 8 hours, though I think that 10 hours is about the normal practice at present. There is a tendency in some portions of the trade, however, to extend the time of leaching considerably. By continuing the extraction for 24 hours or longer very different results are obtained. As 10 hours is about the general practice, I think approximately that figure could be inserted in the method without any difficulty.

Mr. VEITCH. I am heartily in favor of what has been said; in tannery practice 10 hours is sufficient. But the point I want to make, Mr. President, is that it is not wise for us to adopt any change without first trying it.

Mr. STILWELL. I agree to that. Ten hours is the normal practice at present. At the same time I am rather anxious to secure some action prohibiting anything over 24 hours' leaching.

Mr. VEITCH. This discussion will put the matter on a good basis until some action can be taken. The unnecessary prolonging of the extraction should be stopped.

The PRESIDENT. The question is referred to Committee B for consideration as to the advisability of referring the whole matter to the referee for investigation.

The report of Committee A on recommendations, is in order.

REPORT OF COMMITTEE A ON RECOMMENDATIONS (Continued).

B. L. HARTWELL, *Chairman.*

3. NITROGEN.

DETERMINATION OF NITROGEN.

It is recommended—

1. That in the provisional neutral permanganate method the first two lines be changed to read as follows:

Into a 300 cc low form Griffin beaker weigh 2 grams of the sample, if from a mixed fertilizer; if from concentrated material use a quantity containing approximately 0.075 gram of nitrogen.[a]

2. That the study of methods for the determination of available organic nitrogen be continued.

[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. No. 67, p. 77, or Bul. No. 73, p. 49.

Milk and cheese proteids.

It is recommended—

1. That the method given [in the referee's report, page 91] for the separation and determination of casein monolactate and casein dilactate be adopted as a provisional method.

Vegetable proteids.

1. That Mr. Harcourt's report on vegetable proteids be included in the printed proceedings.

Meat proteids.

1. That the following methods be given further trial by the association:

Phosphotungstic acid alone in hot and cold solutions.

Phosphotungstic acid, followed by bromin.

Phosphotungstic acid, followed by zinc sulphate.

Tannin salts, followed by zinc sulphate.

2. That the method for precipitation by bromin alone, and by bromin in the filtrate from zinc sulphate, be discontinued.

4. Soils.

It is recommended—

1. That in the continuation of the work on methods for available phosphoric acid the referee compare greater concentrations than N/200 acid.

2. That the recommendation calling for a continuation of the work on the solubility of phosphoric acid and potash in the different soil layers and for an investigation of methods for determining total phosphoric acid be left to the discretion of the referee.

3. That the method outlined in the referee's report (p. 145) for the analysis of the water-soluble portion of alkali soils be given further study before its adoption as a provisional method.

4. That on page 72, under 4 (a), acid digestion of the soil, for the words "and again evaporate to complete dryness" substitute the following: Filter, wash free of chlorids, and again evaporate to complete dryness as before.

5. That on page 74, under 4 (g), determination of phosphoric acid, the official method given be marked "(a)" and the following optional provisional method be inserted as (b):

(b) *Optional provisional method:* Proceed as in (a) "until all the phosphoric acid is precipitated," and then finish the determination as follows:

After standing for three hours at a temperature not above 50°, filter on a small filter, wash with water until two fillings of the filter do not greatly diminish the color produced with phenolphthalein by one drop of standard alkali. Place the filter and precipitate in the beaker and dissolve in standard alkali, add a few drops of phenolphthalein solution and titrate with standard acid, 1 cc of which equals 0.0005 gram of phosphoric acid (P_2O_5).

6. That on page 74, 4 (h), the word "available" be omitted from the heading "Provisional method for the determination of available phosphoric acid."

7. That on page 76, paragraph 10, determination of humus, the tenth line, the sentence beginning, "The supernatant liquid is filtered, etc.," shall be changed to read as follows: "The supernatant liquid is filtered, and the filtrate must be perfectly clear and free from turbidity; evaporate an aliquot portion, dry at 100°, and weigh."

8. That the retiring referee be instructed to make such verbal corrections in the methods as these changes will involve, and submit them to the secretary for approval and publication.

RESOLUTION.

W. A. WITHERS. *Resolved*, That the referee on soils be requested to test, if possible, the value of proposed methods for comparing the ability of different soils to support the growth of nitrifying organisms when the conditions are identical as to temperature, moisture, number of germs, etc.

5. ASH.

It is recommended—

1. That the method for the determination of sulphates, as described on page 25 of the Proceedings of the Nineteenth Annual Convention of this association, 1902,[a] be printed as a provisional method.

2. That the peroxid method of determining sulphates be subjected to further study, with a view to substituting it for the nitric-acid method.

6. INSECTICIDES.

It is recommended—

1. That Method I for total arsenious oxid in Paris green be adopted as an official method, this being a repetition of last year's recommendation.

Adopted as an official method.

2. That the four modifications of the Avery-Beans method for total arsenic in Paris green be tested to determine which is the best modification to be adopted as an optional official method.

3. That the electrolytic method be adopted as the official method for determining copper in Paris green, and in copper carbonate, and that the volumetric methods be further tested, paying particular attention to the remarks of Messrs. Davidson, McBryde, and Allen upon this subject.[b] (Recommended for the first time.)

4. That an attempt be made to so change the procedure in the analysis of London purple that some of the organic matter may be removed. The remarks of Messrs. Davidson and McBryde are of value in this connection.[b]

5. That the volumetric silver-nitrate method be adopted as the official method of determining cyanogen in potassium cyanid and that an N/20 instead of N/10 solution of silver nitrate be used. (Recommended for the first time.)

6. That the methods of examination of soda lye be further tested and compared with gravimetric methods and that the points brought out by Messrs. Davidson and McBryde be given consideration.[b]

7. That the Kissling method for determining nicotin be adopted as an official method. (Recommended for the first time.)

8. That special attention be given to the methods for determining formaldehyde in order to avoid needless repetition of work.

(The report of Committee A was voted upon by sections and as a whole and adopted.)

The PRESIDENT. The report of Committee B is in order.

REPORT OF COMMITTEE B ON RECOMMENDATIONS.

G. E. PATRICK, *Chairman.*

(Dairy products, foods and feeding stuffs, sugar, and tannin.)

1. DAIRY PRODUCTS.

It is recommended—

1. That the referee on dairy products be requested to continue the investigation of the effects of preservatives upon the albumin of milk.

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[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. No. 73.

[b] See report on insecticides, page 195.

2. Foods and Feeding Stuffs.

It is recommended—

1. That the referee be requested to study the composition of the ether extract obtained after digesting with pepsin the residue obtained by the official method of fat extraction.

2. That the modified König method for the determination of crude fiber be further investigated, especially to ascertain whether the glycerol-acid mixture hydrolyzes any of the true fiber.

3. Sugar.

It is recommended—

1. That the associate referee on chemical methods be instructed to cooperate with the International Association for the Unification of Sugar Methods to the end that a uniform method for the determination of reducing sugars be obtained.

2. That an associate referee on methods of analysis of molasses be appointed.

3. That the following methods and changes in methods be adopted:

a. Changes in the official methods given in paragraph 1, page 56, and paragraph 2, page 57, of the Proceedings of this association for 1902.[a]

Adopted as official.

b. The provisional method given in paragraph (f), page 57, of the Proceedings of the association for 1902

c. Under "Optical methods for the determination of sucrose" omit Clerget's method and Creydt's method (pp. 39 and 40 of Bul. No. 46, Methods of Analysis) and substitute therefor the German official methods for sucrose and raffinose as the official methods of this association. These methods are given on page 57 of the Proceedings of the association for 1902, but in the twenty-fifth line from the top of the page it is recommended to change the words "Dissolve 13.024 grams of the substance in 75 cc of water," so as to read, "Take 50 cc of the clarified solution freed from lead, add 25 cc of water—".

Adopted as official.

d. As an alternate official method for inversion without the application of heat the following is recommended:

Take 50 cc of the clarified solution freed from lead, add 5 cc of hydrochloric acid containing 38.8 per cent of acid, set aside during a period of 24 hours at a temperature not below 20° C.; or if the temperature be above 25° C. set aside for 10 hours. Make up to 100 cc at 20° C. and polarize.

Adopted as official.

e. As provisional methods for the analysis of sugar beets:

Scheibler's alcoholic method and Pellet's aqueous method (p. 58, Proceedings for 1902).

f. As provisional, the methods selected by the international committee for unifying methods of sugar analysis (pp. 58, 59, Proceedings for 1902, pars. 1 to 9, inclusive).

4. That the referees on sugar be requested to continue investigations along the same line as during the past year.

Tannin.

The following recommendations were not received until after Committee B had finished its work, and they were referred by the association to the referee for 1904:

It is recommended—

1. That the last sentence of Paragraph II, Quantity of Material (Bul. 46, Methods of Analysis, p. 79), be changed to read as follows:

In the case of extract, weigh in a weighing bottle, fitted with a ground-glass stopper, such quantity, etc.

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[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. No. 73.

2. That the last sentence of Paragraph V, Soluble Solids (Bul. 46, Methods of Analysis, p. 80), be changed to read as follows:

To guard against loss by evaporation, the flask receiving the filtered liquor shall be stoppered with a rubber stopper and the funnel stem passed through the perforation. The funnel shall be covered with a glass plate having an orifice only sufficiently large to admit the air required by the displacement of the liquor.

A motion made by Mr. Stilwell, and amended by Mr. Veitch, to the effect that the time of extraction of spent tan bark be fixed at 10 hours at steam heat, was referred to Committee B, but no action was taken. Aside from the action taken on the tannin recommendations as noted above, the several sections were acted upon and the report adopted as a whole.

The PRESIDENT. The report of Committee C will be received.

REPORT OF COMMITTEE C ON RECOMMENDATIONS.

A. E. LEACH, *Chairman.*

(Food adulteration.)

1. DISTILLED LIQUORS.

On page 96 of Bulletin No. 65,[a] under Determination of Alcohol, substitute for the first sentence the following: "Weigh or measure (at 15.6° C.) in a distilling flask 20 to 25 cc. of the sample, dilute with 100 cc. of water, and proceed as directed on page 82."

On the same page, under Determination of Extract, strike out the method there given and insert the following: "Weigh or measure (at 15.6° C.) 100 cc of the sample, evaporate nearly to dryness on the water bath, then transfer to a water oven, and dry at the temperature of boiling water."

On the same page, under Determination of Acidity, strike out the method given and insert the following:

Titrate 100 cc (or 50 cc diluted to 100 cc if the sample is dark in color) with decinormal barium hydrate, using phenolphthalein as indicator. The number of cubic centimeters employed is multiplied by 0.006 for the acidity expressed in grams of acetic acid per 100 cc.

On the same page, under the Determination of Fusel Oil, strike out the third paragraph and substitute the following:

Add a small quantity of alkali to 200 cc of the sample under consideration and distill slowly, till about 175 cc have passed over, allow the distilling flask to cool, add 25 cc of water, and distill again until the total distillate measures 200 cc. Dilute the distillate to exactly 30 per cent by volume[b] (sp. gr. 0.96541 at 15.6°).

On page 98 strike out the method given under Determination of Ethereal Salts and substitute the following:

Neutralize the residue left after distillation in the fusel oil determination with N/10 H_2SO_4 and add an excess of 10 cc of the acid. Let stand five minutes and make up to 200 cc. Titrate 2 portions of 25 cc each, using as indicators methyl orange in the first and phenolphthalein in the second. The difference gives the amount of alkali necessary to neutralize the organic acids in 25 cc of the sample. By subtracting from this figure the number of cubic centimeters of alkali required for the free acids, and multiplying the result by 0.0088, the number of drams of ethereal salts (calculated as ethyl acetate) in 25 cc of the sample is determined.

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[a] U. S. Dept. Agr., Bureau of Chemistry, Provisional Methods for the Analysis of Foods.

[b] Refers to footnote *b* on p. 96 of Bul. No. 65.

2. Dairy Products.

On page 36, under Detection of Formaldehyde, designate the method there given as (a) *Hehner's method*, and insert the following method, lettered (b):

(b) *The hydrochloric acid and ferric chlorid method.*[a]—To 10 cc of milk in a porcelain casserole add an equal volume of concentrated hydrochloric acid containing 1 cc of 10 per cent ferric chlorid solution to each 500 cc of acid. Heat nearly to the boiling point over the free flame, holding the casserole by the handle and giving it a rotary motion to break up the curd. A violet coloration indicates formaldehyde.

Insert also as (c) the phloroglucin method as described at the foot of page 72.

On page 36, after section 7, "Detection of Borax and Boric Acid," insert the following:

8. Detection of Benzoic Acid.[b]

Add 5 cc of dilute hydrochloric acid to 50 cc of the milk in a flask and shake to curdle. Then add 150 cc of ether, cork the flask and shake well. Break up the emulsion which forms by aid of a centrifuge, or if the latter is not available extract the curdled milk by gently shaking with successive portions of ether, avoiding the formation of an emulsion.[c] Transfer the ether extract (evaporated to small volume if large in bulk) to a separatory funnel and separate the benzoic acid from the fat by shaking out with dilute ammonia, which takes out the former as ammonium benzoate. Evaporate the ammonia solution in a dish over the water bath till all free ammonia has disappeared, but before getting to dryness add a few drops of ferric chlorid reagent. The characteristic flesh-colored precipitate indicates benzoic acid. Care should be taken not to add the ferric chlorid till all the ammonia has been driven off, otherwise a precipitate of ferric hydrate is formed.

9. Detection of Salicylic Acid.

Proceed exactly as directed for benzoic acid. On applying the ferric chlorid to the solution after evaporation of the ammonia, the well-known violet color indicates salicylic acid when present.[d]

On page 36, "Detection of Foreign Colors" should be numbered as "10." On page 39, change heading 8 to "Detection of Foreign Colors" inserting "(a) *Cornwall's method*"[d] before the method as there given.

On page 39, following Cornwall's method, insert the following:

(b) *Method of the Massachusetts Board of Health for annato.*—Treat 2 or 3 grams of the melted and filtered fat (freed from salt and water) with warm, dilute sodium hydroxid, and after stirring pour the mixture while warm upon a wet filter, using to advantage a hot funnel. If annato is present the filter will absorb the color so that when the fat is washed off by a gentle stream of water the paper will be dyed straw color. It is well to pass the warm alkaline filtrate two or three times through the fat on the filter to insure removal of the color. If, after drying the filter, the color turns pink on application of a drop of stannous chlorid solution the presence of annato is assured.

(c) *Geisler's method for azo colors.*[e]—A few drops of the clarified fat are spread out on a porcelain surface and a pinch of fuller's earth added. In the presence of various azo dyes a pink to violet-red coloration will be produced in a few minutes. Some varieties of the fuller's earth react much more readily than others with azo colors.

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[a] An. Rep. Mass. State Board of Health, 1897, p. 558. Food and Drug Reprint, p. 20.

[b] Leach. An. Rep. Mass. State Board of Health, 1902. Food and Drug Reprint, p. 23.

[c] A volume of ether largely in excess over that of the curdled milk has been found to be less apt to form an obstinate emulsion.

[d] These methods for salicylic and benzoic acids while especially applied to milk, from which the ether extracts both fat and preservative, are useful also with modifications for many other food products. The extraction of the ether solution with dilute ammonia, whereby the preservative is removed, permits the subsequent recovery of the ether by distillation.

[e] J. Am. Chem. Soc., 1898, **20**: 110.

(d) *Low's method for azo colors.*[a]—A small amount of the material to be tested is melted in a test tube, an equal volume of a mixture of one part of concentrated sulphuric acid and four parts of glacial acetic acid is added and the tube is heated nearly to the boiling point, the contents being thoroughly mixed by shaking. The tube is then set aside and after the acid solution has settled out it will be found to be colored wine-red in the presence of azo color, while with pure butter fat comparatively no color will be produced.

(e) *Doolittle's method for azo dyes and annato.*[b]—The melted sample is first filtered. Two test tubes are taken and into each are poured about two grams of the filtered fat which is dissolved in ether. Into one test tube 1 or 2 cc of dilute hydrocloric acid are poured, and into the other about the same volume of dilute potassium hydroxid solution. Both tubes are well shaken and allowed to stand. In the presence of azo dye the test tube to which the acid has been added will show a pink to wine-red coloration, while the potash solution in the other tube will show no color. If annato has been used, on the other hand, the potash solution will be colored yellow while no color will be apparent in the acid solution.

Also on page 39 strike out section 9, Detection of Aniline Colors, and in its place insert the following:

9. Detection of Boric Acid in Butter.

Melt about 25 grams of the sample on the water bath, pour off the fat from the aqueous solution that settles to the bottom of the container, acidify the aqueous solution slightly with hydrochloric acid and test in the usual manner with tumeric paper for boric acid.

On page 40, under Determination of Fat, insert the following heading for the method there given: "(a) *Official method.*[a]"

On page 41, at the end of the first paragraph, insert the following:

(b) *Lythgoe's modification of the Babcock method.*—Weigh accurately about 6 grams of the sample in a tared beaker. Add 10 cc of boiling water and stir with a rod till the cheese softens and an even emulsion is formed, preferably adding a few drops of strong ammonia to aid in the softening and emulsionizing, and keep the beaker in hot water till the emulsion is tolerably complete and free from lumps.

If the sample is a full cream cheese a Babcock cream bottle is employed. The contents of the beaker, after cooling, are transferred to the test bottle as follows: Add to the beaker about half of the 17.6 cc of sulphuric acid regularly used for the test, stir with a rod and pour carefully into the bottle, using the remainder of the acid in two portions for washing out the beaker. Finally proceed as in the Babcock test for milk. Multiply the fat reading by 18 and divide by the weight of the sample taken to obtain the per cent of fat.

On page 41, at the end of the methods for dairy products, insert under the heading "(C) Condensed Milk," the method given on page 28 of the referee's report, 7 sections.

3. Saccharine Products.

On page 48, under Determination of Commercial Glucose in Molasses Sirups and Honey, make the following changes:

At the end of the second paragraph insert "Express results in terms of commercial glucose polarizing at 175°."

Cancel the third paragraph and substitute the following:

In honey, which is composed largely of invert sugar, much more accurate results are attained by polarizing at a temperature of 87° in a water-jacketed tube an inverted, half normal solution of the sample prepared as follows: Weigh out one-half the normal weight of the sample (13.024 grams) in a 100 cc graduated flask, dissolve in about 70 cc of water, and add 7 cc of concentrated hydrochloric acid; then heat to 68° C. and cool in the usual manner. After inversion add a few drops of phenolphthalein and enough sodium hydroxid to neutralize. Discharge the pink color with a few drops of dilute hydrochloric acid, cool again, add from 5 to 10 cc of

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[a] J. Am. Chem. Soc., **20**: 889.
[b] U. S. Dept. Agr., Bureau of Chemistry, Bul. No. 65, p. 152.

alumina cream and make up to the mark. Multiply by 2 the reading at 87° in the 200 mm tube; divide the result by the factor 163 to express the glucose in terms of glucose polarizing at 175°.

Strike out footnote *b*, in the fourth paragraph.

4. Edible Oils and Fats.

On page 26, before heading 5, Determination of Saponification Number and Soluble and Insoluble Acids, insert the Hanus method as a provisional optional method. This method is printed in full in the referee's report, page 63. The principal points in which it differs from the Hübl method are as follows:

Dissolve 13.2 grams of iodin in 1,000 cc glacial acetic acid (showing no reduction with bichromate and H_2SO_4), and add enough bromin to double the halogen content determined by titration. Three cc of bromin is about the proper amount. The iodin may be dissolved by the aid of heat, but the solution should be cold when bromin is added. The solution is used in the same manner as in the Hübl method except that an excess of 70 per cent of the iodin is allowed; 10 cc of the KI solution is added instead of 20 cc and the time of reaction is shortened to thirty minutes. When the KI solution is added, shake the mixture thoroughly before adding the water.

On page 32, under heading 16, Halphen reaction for cotton-seed oil, third line, insert before the word "brine" the word "saturated," and for "fifteen minutes" substitute "an hour to 2 hours."

On page 33, under heading 18, Renard's Test for Peanut Oil, etc., substitute the method printed in full in the referee's report on page 64. This method is essentially the same as that given on page 33, except that 20 grams of fat are taken for analysis with the proportional increase of reagents rendered necessary by the larger amount of fat. An additional washing is also provided for in the revised method.

In Bulletin 46, page 49, under Determination of Refractive Index, second paragraph, line 3, change the factor 0.000176 to 0.000365, in accordance with the more correct factor given in Bulletin 65. Also cancel the example following and substitute the example given on page 22 of Bulletin 65, under Determination of Index of Refraction. (Recommended for the first time.)

5. Spices.

On page 58 of Bulletin 65, under Determination of Starch by the Diastase Method, fifteenth line, strike out the words "copper reduced by" and insert therefor "dextrose resulting from the inversion of."

6. Fruits and Fruit Products.

The following changes in the provisional methods as given in Bulletin 65 are recommended:

(1) On page 78, under heading 12, Polarization, substitute the method given on page 57 of the Proceedings for 1902, under Determination of sucrose in absence of raffinose.

(2) On page 78, section 13, b, line 8, change "1 per cent" to "0.50 per cent."

(3) On page 78, section 14, lines 8 and 9, for "use Allihn's method for the determination (p. 49) *a*," substitute "Use the official method for the determination of invert sugar as given in Bul. 46, p. 33 (c) (1) (a) and (b)."

(4) On page 78, strike out section 15, Determination of Dextrin, and substitute therefor Determination of Glucose, referring to method given on page 48, section 12.

7. Vinegar.

On page 65 of Bulletin 65, following section 13, Detection of Coloring Matters, insert Crampton and Simon's method for the detection of caramel as follows: [a]

Add 25 grams of fuller's earth to 50 cc of the vinegar under examination, beat the mixture up in a beaker and let it stand covered half an hour at room temperature, then filter. The determination of the figure representing the color is made with the tintometer upon the liquid before and after treatment and the difference between the two results gives the percentage of color absorbed.

8. Cocoa and Cocoa Products.

The report submitted is to be printed separately and distributed to the associate referees on food adulteration for criticism before it is printed in the Proceedings, in accordance with the plan previously pursued.

(The recommendations of Committee C were adopted by the association.)

The President. If there is no other business before the association, I will declare the convention adjourned sine die.

Ervin E. Ewell, until recently assistant chief of the Bureau of Chemistry of the Department of Agriculture, and since 1889 a member of that Bureau and of the association, died at New Orleans, La., February 7, 1904, after a brief illness of typhoid fever.

During his fourteen years' service in the Bureau Mr. Ewell accomplished an immense amount of routine work in addition to the research work for which he was especially fitted. These researches included a chemical and physiological study of the mescal button, used by the Mexican Indians, which produces intoxication of a peculiar character; a series of experiments on the nitrifying ferments of soils; miscellaneous studies of a varied character, and investigations of the quality and character of supplies furnished the different branches of the Government, which work has resulted in the establishment of a contracts laboratory in the Bureau of Chemistry. In this work Mr. Ewell's ability was most marked, requiring as it did not only a high degree of chemical skill but also the development of special methods of investigation and great ingenuity in the application of tests.

Mr. Ewell served the association twice as referee on sugar, was vice-president of the Washington section of the American Chemical Society, and had published a number of papers in the journal of that body and in official bulletins of the Department of Agriculture.

In March, 1903, Mr. Ewell resigned from the Bureau of Chemistry to enter a wider field of activity offered by the German Kali Works, with headquarters at Atlanta, Ga., taking with him the best wishes of his associates in the Department. Indefatigable industry, combined with a thorough educational equipment and a fine mentality, had marked Mr. Ewell among the rising young chemists of the day, and science has lost a most promising devotee in his death.

Norman Robinson, formerly an active member of this association, died in Orlando, Fla., on February 4, 1904. Professor Robinson had served the association as referee on potash in 1903, and to his services as a chemist added those of an educator, being an ardent classical scholar as well as a lover of science. Though in astronomy, botany, and geology he was well versed, it was to chemistry that he gave the most attention, working in his private laboratory in Jacksonville in addition to his professional duties. The association has lost in his death a most able and scholarly member of the profession, and to his family all those who remember Professor Robinson will extend their sympathy in the loss of his companionship. High principles, a mind at once keen and broadly cultured, and a charming personality made Professor Robinson a man to be long and lovingly remembered by those who knew him even slightly.

OFFICERS, REFEREES, AND COMMITTEES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR 1904.

President.

Mr. M. E. Jaffa, Berkeley, Cal.

Vice-President.

Mr. C. L. Penny, Newark, Del.

Secretary.

Mr. H. W. Wiley, Washington, D. C.

Additional members of Executive Committee.

Mr. W. P. Headden, Fort Collins, Colo.
Mr. W. R. Perkins, Agricultural College, Miss.

Referees.

Phosphoric acid: C. B. Williams, Raleigh, N. C.
Nitrogen:
Determination of nitrogen: C. H. Jones, Burlington, Vt.
Separation of nitrogenous bodies: E. B. Hart, Geneva, N. Y. (milk and cheese proteids).
Potash: F. B. Carpenter, Richmond, Va.
Soils: C. G. Hopkins, Urbana, Ill.
Dairy products: G. E. Patrick, Washington, D. C.
Foods and feeding stuffs: J. O. La Bach, Lexington, Ky.
Food adulterations: W. D. Bigelow, Washington, D. C.
Sugar: L. S. Munson, Washington, D. C.
Tannin: George A. Kerr, Damascus, Va.
Insecticides: B. H. Smith, Washington, D. C.
Ash: R. W. Thatcher, Pullman, Wash.
Medicinal plants and drugs: L. F. Kebler, Washington, D. C.

Associate referees.

Phosphoric acid: F. P. Veitch, Washington, D. C.
Nitrogen:
Determination of nitrogen: F. A. Urner, Geneva, N. Y.
Separation of nitrogenous bodies:
Meat proteids: W. D. Bigelow, Washington, D. C.
Vegetable proteids: J. S. Chamberlain, Washington, D. C.
Potash: G. S. Fraps, College Station, Tex.
Soils: R. H. Loughridge, Berkeley, Cal.
Dairy products: F. W. Woll, Madison, Wis.
Foods and feeding stuffs: J. K. Haywood, Washington, D. C.

Food adulterations:

(1) Colors: W. G. Berry, Appraiser's Office, New York, N. Y.
(2) Saccharine products, including confectionery: E. B. Kenrick, Winnipeg, Manitoba, Canada.
(3) Fruit products: E. M. Chace, Washington, D. C.
(4) Wine: G. E. Colby, Berkeley, Cal.
(5) Beer: H. E. Barnard, Concord, N. H.
(6) Distilled liquors: C. A. Crampton, Washington, D. C.
(7) Vinegar: Richard Fischer, Madison, Wis.
(8) Flavoring extracts: R. E. Doolittle, Lansing, Mich.
(9) Spices: A. L. Winton, New Haven, Conn.
(10) Baking powder and baking chemicals: R. O. Brooks, Trenton, N. J.
(11) Meat and fish: M. E. Jaffa, Berkeley, Cal.
(12) Fats and oils: L. M. Tolman, Washington, D. C.
(13) Dairy products: A. E. Leach, Boston, Mass.
(14) Cereal products: A. McGill, Ottawa, Canada.
(15) Infants and invalids' foods: H. W. Wiley, Washington, D. C.
(16) Vegetables: F. W. Bedford, St. Paul, Minn.
(17) Condiments other than spices: J. D. Hird, Washington, D. C.
(18) Cocoa and cocoa products: E. N. Eaton, Chicago, Ill.
(19) Tea and coffee: H. C. Lythgoe, Boston, Mass.
(20) Preservatives: W. G. Bigelow, Washington, D. C.

Sugar:

Molasses methods: H. E. Sawyer, Boston, Mass.
Special analytical methods: C. A. Browne, jr., Audubon Park, La.

Tannin: H. C. Reed, Stamford, Conn.

Insecticides: S. Avery, Lincoln, Nebr.

Ash: F. T. Shutt, Ottawa, Canada.

CONSTITUTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.

(1) This association shall be known as the Association of Official Agricultural Chemists of the United States. The objects of the association shall be (1) to secure uniformity and accuracy in the methods, results, and modes of statement of analysis of fertilizers, soils, cattle foods, dairy products, and other materials connected with agricultural industry; (2) to afford opportunity for the discussion of matters of interest to agricultural chemists.

(2) Analytical chemists connected with the United States Department of Agriculture, or with any State or national agricultural experiment station or agricultural college, or with any State or national institution or body charged with official control of the materials named in section 1, shall alone be eligible to membership; and one such representative for each of these institutions or boards, when properly accredited, shall be entitled to enter motions or vote in the association. Only such chemists as are connected with institutions exercising official fertilizer control shall vote on questions involving methods of analyzing fertilizers. All persons eligible to membership shall become members ex officiis and shall be allowed the privileges of membership at any meeting of the association after presenting proper credentials. All members of the association who lose their right to such membership by retiring from positions indicated as requisite for membership shall be entitled to become honorary members and to have all privileges of membership save the right to hold office and vote. All analytical chemists and others interested in the objects of the association may attend its meetings and take part in its discussions, but shall not be entitled to enter motions or vote.

(3) The officers of the association shall consist of a president, a vice-president, and a secretary, who shall also act as treasurer; and these officers, together with two other members to be elected by the association, shall constitute the executive committee. When any officer ceases to be a member by reason of withdrawing from a department or board whose members are eligible to membership, his office shall be considered vacant, and a successor may be appointed by the executive committee, to continue in office till the annual meeting next following.

(4) There shall be appointed by the executive committee, at the regular annual meeting, a referee and such associate referees for each of the subjects to be considered by the association as that committee may deem appropriate.

It shall be the duty of these referees to prepare and distribute samples and standard reagents to members of the association and others desiring the same, to furnish blanks for tabulating analyses, and to present at the annual meeting the results of work done, discussion thereof, and recommendations of methods to be followed.

(5) The special duties of the officers of the association shall be further defined, when necessary, by the executive committee.

(6) The annual meeting of this association shall be held at such place as shall be decided by the association, and at such time as shall be decided by the executive committee, and announced at least three months before the time of meeting.

(7) No changes shall be made in the methods of analysis used in official inspection, except by unanimous consent, until an opportunity shall have been given all official chemists having charge of the particular inspection affected to test the proposed changes.

(8) Special meetings shall be called by the executive committee when in its judgment it shall be necessary, or on the written request of five members; and at any meeting, regular or special, seven enrolled members entitled to vote shall constitute a quorum for the transaction of business.

(9) The executive committee will confer with the official boards represented with reference to the payment of expenses connected with the meetings and publication of the proceedings of the association.

(10) All proposed alterations or amendments to this constitution shall be referred to a select committee of three at a regular meeting, and after report from such committee may be adopted by the approval of two-thirds of the members present entitled to vote.

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